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Characteristics of Patients with Prostate Cancer Who Have Initially been Treated by Hormone Therapy in Japan: J-CaP Surveillance

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Objective: Hormone therapy for prostate cancer has empirically prevailed in Japan. We planned to evaluate the trends and outcome of hormone therapy for establishing an adequate guideline.
Methods: Patients with prostate cancer who were initially treated by hormone therapy were registered through the J-CaP registration system. This report summarizes the background factors.
Results: From January 2001 to October 2003, 17 872 patients were registered from 395 institutes throughout Japan. The background factors of 17 312 patients were analyzed. The 17 872 patients were estimated as composing more than half of newly diagnosed prostate cancer patients in Japan. Of these, 22.9, 35.1, 32.9 and 8.6% belonged to T1, T2, T3 and T4, respectively. For the purposes of hormone therapy, 77.5% was primary hormone therapy. Neoadjuvant setting and adjuvant setting were 18.1 and 4.3%, respectively. About 60% of the hormone therapy was combined hormone therapy with LH-RHa plus anti-androgens.
Conclusion: Irrespective of patients' age, TNM, stage of illness, or histological background, the majority of prostate cancer patients in Japan are receiving hormone therapy. It is necessary to evaluate whether this trend is merely a continuation of past experience of Japanese urologists or if there is a difference in the profile of effect and side-effect in the case of Japanese patients compared to therapy given in Westerners.

Key words: prostate cancer – hormone therapy – endocrine therapy

INTRODUCTION

In prostate cancer treatment, hormone therapy has been used in Europe and North America mainly to provide temporary relief for advanced cancers. However, the CaPSURE report (1), released in 2003, indicates that there is a rapid increase in the use of hormone therapy on localized cancer in the United States, which suggests a drastic change in the role of hormone therapy. Meanwhile, in Japan, hormone therapy has been used over many years in a considerable number of patients with localized or locally advanced prostate cancer. In recent years, while clinical trial data (2,3) indicating its usefulness have been accumulating, the outcomes have yet to be accurately analyzed. As typically seen in the early prostate cancer (EPC) studies of recent years in Europe and North America (4), clinical trials are being reported that point to the effectiveness of hormone therapy in localized cancer (5,6). Against this backdrop, in 2001 the Japan Study Group of Prostate Cancer (J-CaP Study Group) was inaugurated with financial support from the Japan Kidney Foundation. This project has been authorized by the Japan Urological Association. The purposes of this study group were to gather information about the hormone therapy administered to Japanese prostate cancer patients living in Japan and to analyze the outcomes of treatment in order to create a guideline for optimal hormone therapy. This report summarizes the background factors of patients receiving hormone therapy across most of Japan.

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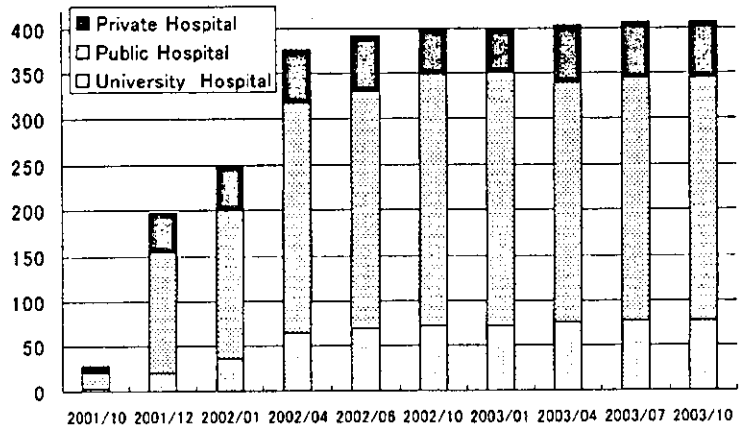

Figure 1. Overview of the year of registration and type of institution.

PATIENTS AND METHODS

The rules for the J-CaP study group are summarized in the Appendix.

ELIGIBLE INSTITUTIONS

Eligible institutions are Japanese urological institutions endorsing the purpose of this study that are able to obtain the approval of their own ethics committees (or IRB). Institutions that have not yet established their own ethics committee (or IRB) but can be vetted instead by an affiliated institution or can obtain approval from the person responsible for the institution are also included. As a rule, in each eligible institution, all cases of patients newly starting hormone therapy for prostate cancer in and after January 2001 will be regarded as subjects of the study.

PERIOD OF RESEARCH

Registration will commence when approval is obtained from the J-CaP Study Group. The term of case registration is for 3 years and the follow-up period is for 2 years.

METHOD

Data under the following headings for each registered case will be relayed to the secretariat server over the Internet: date of birth, family history, date of PSA reading, PSA value, PSA kit name, testosterone value, biopsy date, Gleason score, histological grade, clinical stage, case history, details of hormone therapy, whether or not there has been progress observation, whether or not surgery was carried out, date of surgery, operative procedure, whether or not radiotherapy is being conducted, irradiation method, irradiation date, progress. TNM classification used was the 5th edition (7). Histological grade and other criteria were adopted in accordance with the Japanese Urological Association/Japan Society of Pathology 3rd Edition of General Rules for Clinical and Pathological Studies on Prostate Cancer (8).

FOLLOW-UP METHOD

The registered cases, as a rule, are to be updated once every 3 months with regard to test data, change in treatment and progress data. The secretariat immediately contacts institutions not updating information, requesting data input. The secretariat forwards input forms for data addition, and confirms registered cases as of that date as necessary. Additionally, assistance can be given on adding test data and entering changes in treatment and progress data.

This report concerns patient background factors, tumor factors and treatment details of registered cases between 2001 and October 2003.

RESULTS

PARTICIPATING INSTITUTIONS

By October 2003, 395 institutions throughout Japan had registered, acquiring IDs and passwords. Eleven institutions of the 395 later withdrew registration. Fig. 1 gives an overview of the year of registration and type of institution. The number of university hospitals registering was 76 (60.2% of university hospitals in Japan); in detail, 35 national university hospitals (83.3%) have been included.

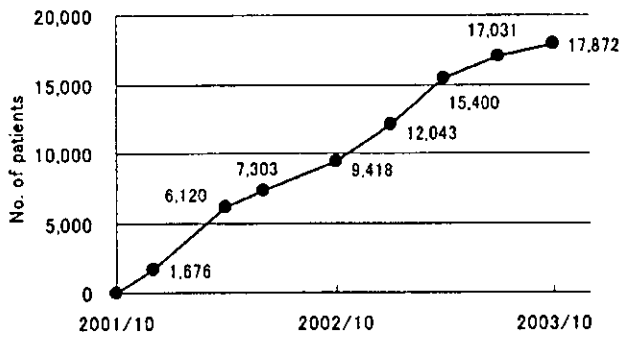

Figure 2. Cumulative number of patients registered.

Table 1. Patient backgrounds; family history of prostate cancer, age at diagnosis and PSA value at diagnosis

	2001	2002	2003	Total	%
Family history					
No history	5959	6104	1771	13 834	79.9
Within 2nd degree of relationship	120	128	29	277	1.6
Within 3rd degree of relationship	11	8	3	22	0.1
Don't know	1412	1453	314	3179	18.4
Total	7502	7693	2117	17 312	100.0
Age at diagnosis					
<60	329	320	61	710	4.1
60–64	596	620	161	1377	8.0
65–69	1197	1265	331	2793	16.1
70–74	1935	2037	567	4539	26.2
75–79	1798	1889	562	4249	24.5
≥80	1647	1562	435	3644	21.0
Total	7502	7693	2117	17 312	100.0
PSA at diagnosis					
<4	255	269	73	597	3.4
4–<10	1680	1863	556	4099	23.7
10–<20	1470	1628	493	3591	20.7
20–<50	1459	1514	387	3360	19.4
≥50	2612	2401	606	5619	32.5
No description	26	18	2	46	0.3
Total	7502	7693	2117	17 312	100.0

Number of Registered Patients

As shown in Fig. 2, 17 872 patients were registered by October 2003. This survey investigated patients who were first diagnosed with prostate cancer at the registered institutions during this period. Respectively, 7952 and 8195 new patients were reported in 2001 and 2002 by 246 and 216 institutions. Of these new patients, 5969 and 6064 were newly administered hormone therapy, and 5646 and 5651 were registered with J-CaP. In summary, it is shown that 75% of new patients were given hormone therapy in some form and 70% registered with J-CaP.

Patient Background Factors

Of the 17 872 registered patients at the time of data compilation, data were collected from 17 312 patients. 529 cases without any record of hormone therapy commencement date were excluded, as were 31 cases whose therapy was reported as commencing in 2000. Family history, age at diagnosis and PSA value at diagnosis are given in Table 1.

Tumor Background Factors

A summary of Gleason score, histological grade, TNM classification and clinical stage (TNM) is given in Table 2.

Table 2. Tumor backgrounds; Gleason score, histological grade, TNM classification, TNM clinical stage

	2001	2002	2003	Total	%
Gleason score					
2–4	654	551	150	1355	7.8
5	696	744	251	1691	9.8
6	1029	1250	401	2680	15.5
7	1595	1958	579	4132	23.9
8–10	1801	2337	590	4728	27.3
No description	1727	853	146	2726	15.7
Total	7502	7693	2117	17 312	100.0
Histological differentiation					
Well	1489	1554	453	3496	20.2
Moderate	3360	3362	990	7712	44.5
Poor	1995	1997	513	4505	26.0
Unknown	103	119	16	238	1.4
No description	555	661	145	1361	7.9
Total	7502	7693	2117	17 312	100.0
T stage					
T0	1	3	0	4	0.0
T1	1630	1813	518	3961	22.9
T2	2566	2680	832	6078	35.1
T3	2597	2509	589	5695	32.9
T4	673	657	157	1487	8.6
Tx	27	25	12	64	0.4
No description	8	6	9	23	0.1
Total	7502	7693	2117	17 312	100.0
N factor					
N0	6000	6315	1767	14 082	81.3
N1	1004	917	210	2131	12.3
Nx	462	427	119	1008	5.8
No description	36	34	21	91	0.5
Total	7502	7693	2117	17 312	100.0
M factor					
M0	5380	5696	1634	12 710	73.4
M1	157	119	12	288	1.7
M1a	83	77	11	171	1.0
M1b	1496	1428	327	3251	18.8
M1c	100	71	19	190	1.1
Mx	250	268	93	611	3.5
No description	36	34	21	91	0.5
Total	7502	7693	2117	17 312	100.0
Clinical stage					
II	3684	3987	1188	8859	51.2
II	1273	1327	326	2926	16.9
IV	2082	1945	444	4471	25.8
No description	463	434	159	1056	6.1
Total	7502	7693	2117	17 312	100.0

Table 3. Purpose of hormone therapy

	2001	2002	2003	Total	%
Hormonal therapy					
Main	5926	5914	1585	13 425	77.5
Adjuvant	306	366	81	753	4.3
Neoadjuvant	1270	1413	451	3134	18.1
Total	7502	7693	2117	17 312	100.0
Hormonal therapy detail					
Orchiectomy only	236	214	63	513	3.0
Orchiectomy + medication	605	427	96	1128	6.5
LH-RHa only	826	1065	319	2210	12.8
LH-RHa + anti-androgen	4431	4703	1249	10 383	60.0
Anti-androgen only	392	584	251	1227	7.1
Other	1012	700	139	1851	10.7
Total	7502	7693	2117	17 312	100.0

Hormone Therapy

As to the reason for hormone therapy, primary application of hormone therapy was the most prevalent, comprising 77.5% of the total, followed by 18.1% neoadjuvant and 4.3% adjuvant (Table 3).

Table 3 also indicates an overview of the types of hormone therapy. The combined use of LH-RHa + anti-androgen drug is the largest, comprising 60%. Anti-androgen monotherapy was 7.1% and LH-RHa monotherapy was 12.8%.

Table 4 shows the relations between the purpose of hormone therapy and T category, clinical stage, Gleason score and age. A notable feature is that in all categories, primary use of hormone therapy was the most common.

Table 5 shows the relations between the type of hormone therapy and T category, clinical stage, Gleason score and age. In all categories and ages, combined androgen blockade (CAB) was used in the main. In Table 6, details are given of the main treatment methods when hormone therapy was administered as neoadjuvant, as well as the details of main treatment methods when used as adjuvant.

Compliance of Survey Data

Omission of data entry among registered data included 0.2% of patients for whom PSA values were not recorded. Meanwhile, omission of histological grade accounted for 7.8% and omission of clinical stage 6.1%. As for Gleason score, 23% of registered cases in 2001 had no entry, but in 2002 this had decreased to 11.9% and by 2003, to 6.5%. This is thought to be because in the First Edition of the Japanese Urological Association and Japan Society of Pathology's General Rules for Clinical and Pathological Studies on Prostate Cancer, Gleason score entry was not compulsory. Only in the Second Edition did Gleason score become required.

Table 4. Relations between the purpose of hormone therapy and T category, TNM clinical stage, Gleason score and patient age

	Main	Adjuvant	Neoadjuvant	Total	%
T stage					
T0	4 (0.1%)			4	0.0
T1	2689 (67.9%)	218 (5.5%)	1054 (26.6%)	3961	22.9
T2	4260 (70.1%)	333 (5.5%)	1485 (24.4%)	6078	35.1
T3	4965 (87.2%)	174 (3.1%)	556 (9.8%)	5695	32.9
T4	1425 (95.8%)	26 (1.7%)	36 (2.4%)	1487	8.6
Tx	60 (93.8%)	2 (3.1%)	2 (3.1%)	64	0.4
No description	22 (95.7%)		1 (4.3%)	23	0.1
Total	13 425 (77.5%)	753 (4.3%)	3134(18.1%)	17 312	100.0
Clinical stage					
II	5847 (66.0%)	537 (6.1%)	2475 (27.9%)	8859	51.2
III	2263 (77.3%)	145 (5.0%)	518 (17.7%)	2926	16.9
IV	4362 (97.6%)	44 (1.0%)	65 (1.5%)	4471	25.8
No description	953 (90.2%)	27 (2.6%)	76 (7.2%)	1056	6.1
Total	13 425 (77.5%)	753 (4.3%)	3134 (18.1%)	17 312	100.0
Gleason score					
2–4	996 (73.5%)	69 (5.1%)	290 (21.4%)	1355	7.8
5	1214 (71.8%)	91 (5.4%)	386 (22.8%)	1691	9.8
6	1902 (71.0%)	120 (4.5%)	658 (24.6%)	2680	15.5
7	3179 (76.9%)	175 (4.2%)	778 (18.8%)	4132	23.9
8–10	3966 (83.9%)	185 (3.9%)	577 (12.2%)	4728	27.3
Unknown	2168 (79.5%)	113 (4.1%)	445 (16.3%)	2726	15.7
Total	13 425 (77.5%)	753 (4.3%)	3134 (18.1%)	17 312	100.0
Age at diagnosis					
<60	364 (51.3%)	48 (6.8%)	298 (42.0%)	710	4.1
60–64	767 (55.7%)	95 (6.9%)	515 (37.4%)	1377	8.0
65–69	1613 (57.8%)	234 (8.4%)	946 (33.9%)	2793	16.1
70–74	3305 (72.8%)	226 (5.0%)	1008 (22.2%)	4539	26.2
75–79	3808 (89.6%)	116 (2.7%)	325 (7.6%)	4249	24.5
≥80	3568 (97.9%)	34 (0.9%)	42 (1.2%)	3644	21.0
Total	13 425 (77.5%)	753 (4.3%)	3134 (18.1%)	17 312	100.0

Follow-up Data

For approximately 92% of the registered cases in 2001 and 75% of the registered cases in 2002, the input of follow-up data was confirmed at least once. The period (median) from the start of hormone therapy to the latest follow-up data entry was 406 days (between 0 and 964) for 2001-registered cases and 189 (between 0 and 615) for 2002-registered cases.

DISCUSSION

In Japan, the General Rules for Clinical and Pathological Studies on Prostate Cancer issued by the Japanese Urological Association and Japan Society of Pathology were first published in

Table 5. Relations between the type of hormone therapy and T category, TNM clinical stage, Gleason score and patient age

	Orchiectomy only	Orchiectomy + medication	LH-RHa only	LH-RHa + anti-androgen	Anti-androgen only	Other	Total	%
T stage								
T0				1 (25.0%)	1 (25.0%)	2 (50.0%)	4	0.0
T1	112 (2.8%)	169 (4.3%)	719 (18.2%)	2333 (58.9%)	427 (10.8%)	201 (5.1%)	3961	22.9
T2	158 (2.6%)	277 (4.6%)	921 (15.2%)	3737 (61.5%)	532 (8.8%)	453 (7.5%)	6078	35.1
T3	196 (3.4%)	466 (8.2%)	490 (8.6%)	3513 (61.7%)	215 (3.8%)	815 (14.3%)	5695	32.9
T4	44 (3.0%)	208 (14.0%)	69 (4.6%)	752 (50.6%)	46 (3.1%)	368 (24.7%)	1487	8.6
Tx	1 (1.6%)	5 (7.8%)	8 (12.5%)	36 (56.3%)	4 (6.3%)	10 (15.6%)	64	0.4
No description	2 (8.7%)	3 (13.0%)	3 (13.0%)	11 (47.8%)	2 (8.7%)	2 (8.7%)	23	0.1
Total	513 (3.0%)	1128 (6.5%)	2210 (12.8%)	10 383 (60.0%)	1227 (7.1%)	1851 (10.7%)	17 312	100.0
Clinical stage								
II	262 (3.0%)	360 (4.1%)	1527 (17.2%)	5366 (60.6%)	841 (9.5%)	503 (5.7%)	8859	51.2
III	111 (3.8%)	157 (5.4%)	325 (11.1%)	1959 (67.0%)	135 (4.6%)	239 (8.2%)	2926	16.9
IV	115 (2.6%)	559 (12.5%)	246 (5.5%)	2449 (54.8%)	132 (3.0%)	970 (21.7%)	4471	25.8
No description	25 (2.4%)	52 (4.9%)	112 (10.6%)	609 (57.7%)	119 (11.3%)	139 (13.2%)	1056	6.1
Total	513 (3.0%)	1128 (6.5%)	2210 (12.8%)	10 383 (60.0%)	1227 (7.1%)	1851 (10.7%)	17 312	100.0
Gleason score								
2–4	31 (2.3%)	54 (4.0%)	187 (13.8%)	820 (60.5%)	157 (11.6%)	106 (7.8%)	1355	7.8
5	65 (3.8%)	91 (5.4%)	247 (14.6%)	1032 (61.0%)	152 (9.0%)	104 (6.2%)	1691	9.8
6	80 (3.0%)	146 (5.4%)	468 (17.5%)	1579 (58.9%)	241 (9.0%)	166 (6.2%)	2680	15.5
7	151 (3.7%)	247 (6.0%)	557 (13.5%)	2515 (60.9%)	267 (6.5%)	395 (9.6%)	4132	23.9
8–10	119 (2.5%)	445 (9.4%)	373 (7.9%)	2796 (59.1%)	232 (4.9%)	763 (16.1%)	4728	27.3
Unknown	67 (2.5%)	145 (5.3%)	378 (13.9%)	1641 (60.2%)	178 (6.5%)	317 (11.6%)	2726	15.7
Total	513 (3.0%)	1128 (6.5%)	2210 (12.8%)	10 383 (60.0%)	1227 (7.1%)	1851 (10.7%)	17 312	100.0
Age at diagnosis								
<60	5 (0.7%)	32 (4.5%)	76 (10.7%)	413 (58.2%)	65 (9.2%)	119 (16.8%)	710	4.1
60–64	11 (0.8%)	88 (6.4%)	176 (12.8%)	816 (59.3%)	120 (8.7%)	166 (12.1%)	1377	8.0
65–69	57 (2.0%)	175 (6.3%)	319 (11.4%)	1674 (59.9%)	239 (8.6%)	329 (11.8%)	2793	16.1
70–74	96 (2.1%)	248 (5.5%)	564 (12.4%)	2826 (62.3%)	300 (6.6%)	505 (11.1%)	4539	26.2
75–79	153 (3.6%)	302 (7.1%)	566 (13.3%)	2556 (60.2%)	259 (6.1%)	413 (9.7%)	4249	24.5
≥80	191 (5.2%)	283 (7.8%)	509 (14.0%)	2098 (57.6%)	244 (6.7%)	319 (8.8%)	3644	21.0
Total	513 (3.0%)	1128 (6.5%)	2210 (12.8%)	10 383 (60.0%)	1227 (7.1%)	1851 (10.7%)	17 312	100.0

1985 (9) and this set of rules has been widely used ever since. The document gives a guideline on diagnosis and a detailed description of rules associated with making entries on patient background, tumor background and treatment method. Most of the papers presented at such meetings, such as the academic conference of the Urological Association, follow these rules and their diffusion rate is extremely high. The J-CaP survey basically followed the rules, and the accuracy of TNM diagnoses and clinical stage diagnoses is considered to be high. The Japanese Urological Association started a prostate cancer registration system from 2001, in accordance with these rules. However, this system is a registration of all prostate cancers. Therefore, when, for example, focusing on hormone therapy, we cannot necessarily expect satisfactory outcome data.

The morbidity of prostate cancer in Japan has been remarkably lower than in Europe and North America (10). Furthermore, due to anxieties about radiotherapy and the slowness of the introduction of technical expertise in radical prostatectomy, in many cases surgical castration or estrogen administration has been conducted across the board (11). However, in recent years Japan has seen an overwhelming increase in morbidity and mortality from prostate cancer (10). Compounding this, the influx of information about prostate and surgical techniques from Europe and North America has led to a rapidly growing debate on the method of treatment. Naturally, the trend towards newer treatment is beginning with reference to European (12) and North American guidelines (13) and the trend is set to continue.

Table 6. Main treatment for adjuvant or neoadjuvant hormone therapy

	Method	2001	2002	2003	Total
Operation					
Hormonal therapy followed by surgery	Retropubic	1609	18		1627
	Laparoscopic	23			23
	Perineal	17			17
	Other	3			3
	Total	1652	18		1670
Surgery followed by hormonal therapy	Retropubic	256	3		259
	Laparoscopic	10			10
	Perineal	2			2
	Other	2			2
	Total	270	3		273
Irradiation					
Hormonal therapy followed by irradiation	External beam	647	468	69	1184
	External + brachytherapy	15	4		19
	Brachytherapy	12	6	1	18
	Other	11	6	1	118
	Total	685	484	71	1339
Irradiation followed by hormonal therapy	External beam	46	62	10	118
	External + brachytherapy	3			3
	Brachytherapy	1	3		4
	Other	1	1		2
	Total	51	66	10	127

At present, with financial assistance from the Ministry of Health, Labor and Welfare, the Japanese Urological Association is working on the drafting of a prostate cancer treatment guideline at the earliest possible date. What is of concern here is that, in addition to the circumstances previously mentioned, there have been very few clinical trials with strong evidence carried out in this country. This causes a desperate lack in clinical data specific to Japan, which is essential to establish such a guideline. Hormone therapy in Japan, which has been administered only empirically, should be re-examined correctly to determine what outcome it is actually providing for the patients. Otherwise, it is likely that Japan's treatment guideline will become a reproduction of those of Europe and North America. Ethnic and philosophical differences, religious background, differences in perceptions about sex, and economic background—these diverse factors must be taken into account in the drafting of the most appropriate guideline for a country. The general attitude toward hormone therapy in Japan is similar to other East Asian countries (14). The recent treatment and clinical trial findings on hormone therapy in Europe and North America aimed at achieving long-term stable results indicate that we should examine the outcome of hormone therapy not only in Japan but throughout the world (4–6). The CaPSURE data reported in 2003 (1) consists of the analyses of 3439 cases, showing that the proportion of primary hormone treatment on localized prostate cancer rose dramatically from 4.6% in 1989 to 14.2% in 2001 and pointed firmly to the need to review the existing guidelines.

The institutions registered with J-CaP cover 60.2% of all university hospitals. According to Japan Cancer Statistics 2003, the number of patients newly diagnosed with prostate cancer in 1998 was 15 814 (15). In view of the proportion of J-CaP registered patients obtained in the survey of new patient numbers mentioned earlier, ~50% of new prostate cancer patients were treated by hormone therapy and registered with J-CaP. J-CaP had requested reports on the number of newly diagnosed prostate cancer patients in the registered institutions. Out of 358 institutions, 246 had responded as of 2001. Based on this report, 7952 patients were newly diagnosed with prostate cancer in those 246 institutions. Of these, 5969 patients (75.1%) were treated by hormone therapy in some form. Among those patients, 5646 (71%) were registered with J-CaP. In other words, 94.6% of the patients who had initiated a hormone therapy in 2001 were registered with J-CaP. This figure is almost the same in 2002. This illustrates the breadth of significance of this study. Patient background factors and PSA values at diagnosis would not represent the general trend because of the bias that patients registered for this study are receiving hormone therapy for the first time. However, we should make a special note of the low frequency of familial prostate cancer.

For the same reason, the background to the tumor in this report would not represent the overall trend of prostate cancer in Japan. Nevertheless, considering the finding that an extremely large number of patients are receiving hormone therapy, we can safely say that they express the overall background factors of prostate cancer in Japan to a fairly high degree of accuracy.

The analysis of the purpose and types of hormone therapy shows that there is a distinctively different trend in Japan compared to Europe or North America. These are the first findings in Japan based on a large-scale organized survey. To summarize: (i) many patients are receiving hormone therapy irrespective of age, TNM, stage of illness or histological background; (ii) more than 70% of them are under primary hormone therapy; and (iii) roughly 60% undergo combined androgen blockade (CAB). Since no clear outcome investigation has yet been carried out, we should evaluate this present status of hormone therapy in Japan either as: (i) it is merely a continuation of past experience, and in the near future, it should be managed carefully by adopting European and American guidelines; or (ii) it is still difficult to judge whether the effect of hormone therapy for Japanese patients is different in the profile of effects and side-effects from that for Westerners. What is more, in T2 treatment no accurate randomized study has been conducted so far globally on whether surgical treatment and radiotherapy are truly more effective than hormone therapy. Therefore, on this point we must reserve any conclusions.

The NCI-PDQ (13) and EAU guidelines (12) attach virtually no significance to hormone therapy on T2 prostate cancer. As for T3, the emphasis is on its significance as neoadjuvant before radiotherapy and little importance is assigned to the sole application of hormone therapy. Even when there is metastasis, there is debate on whether immediate hormone therapy is appropriate and also on whether there is any point in CAB; however, no clear conclusions have been reached (16,17).

In such circumstances, there are two clinical trial results in Japan reported recently that are extremely interesting. The first (2) is the results of a randomized study on hormone therapy given to localized or locally advanced prostate cancer. This was a comparative trial of LH-RHa + chlormadinone acetate (CMA) versus LH-RHa alone on patients in whom radical prostectomy was not chosen as treatment for whatever reason. The results are interim, with an observation period less than 5 years. So far, progression-free survival is good for CAB. Even when both groups are put together, it has been determined that the same survival rate as the one expected for the population of that age group has been obtained. The other study (3) is a comparative trial of LH-RHa + bicalutamide versus LH-RHa + placebo administered for patients with locally advanced or metastatic prostate cancer. The observational period is again short, but in both PSA progression-free survival and time to PSA response, the CAB group was significantly better. Meanwhile, in a successive survey of QOL using FACT-P that was officially translated into Japanese (18), the CAB group showed a significantly better result (19). This is indicative of the perception that the effects of hormone therapy on QOL are different between Japanese and Western patients (20). Therefore, it is important to examine whether or not recent clinical trial results take into account ethnic differences in the broad sense, including the lifestyle and philosophical backgrounds of Japanese and Western people.

In future, in the treatment of prostate cancer in Japan, it is evident that the importance of hormone therapy should be investigated with specific focus on Japanese people. We await the further analysis of the outcome findings, which is the aim of the J-Cap Study.

APPENDIX

J-CaP Home Page: Rules for Use

1. The J-CaP Home Page is to be created as an Internet server.
2. Use of the case database on the J-CaP Home Page is restricted to doctors who are joint researchers and the use of the database requires a user ID and password issued by means of prior registration.
3. Communication between the case database server and users is to be protected by encryption (SSL).
4. The names of institutions and patients (initials) displayed in the case database are to be encoded so that individual patients cannot be identified.
5. Information concerning joint researchers' institutions and patient names (initials) will only be accessible to database administrators with a special ID and password and only at the designated location (administrative secretariat).
6. The ID and password of the above-mentioned administrators will be stored as strictly confidential and no record of them will be kept.
7. The disposal of case data and information concerning joint researcher institutions and patient names (initials) after the completion of the J-CaP Study Group's research period will be determined at a later date by administrators.

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A retrospective study of the treatment of locally advanced prostate cancer by six institutions in eastern and north-eastern Japan

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OBJECTIVE

To investigate patients with locally advanced prostate cancer treated at six academic institutions in eastern and north-eastern Japan from 1988 to 2000, to facilitate the establishment of Japanese guidelines for the diagnosis and treatment of locally advanced prostate cancer.

PATIENTS AND METHODS

The study included 391 eligible patients with locally advanced prostate cancer who were treated by radical prostatectomy (RP), radiotherapy and/or primary hormone therapy. Disease-specific survival rates for these patients were assessed in relation to their clinicopathological characteristics and the types of treatment they received. The Mann–Whitney *U*-test, Kruskal–Wallis, chi-square and log-rank test were used for statistical analysis, as appropriate.

RESULTS

In all, 128 patient with lower prostate-specific antigen levels ($P = 0.023$) and/or better performance status ($P = 0.001$) had RP. Neoadjuvant hormone therapy before RP was the treatment in 68 (53%) of these 128 patients; 66 (52%) received immediate adjuvant hormone therapy. Of 87 patients treated with radiotherapy, 75 (86%) had external beam radiotherapy (EBRT) as the primary treatment with no brachytherapy, and 12 (14%) had brachytherapy as the primary method. Neoadjuvant hormone therapy was given to 56 of the 87 patients (64%); 48 (55%) received immediate adjuvant hormone therapy. Of the 176 patients treated with primary hormone therapy alone, combined androgen blockade and surgical or medical castration was the treatment in 76 (43%) and 85 (48%), respectively. Disease-specific survival rates at 5 years for patients treated with RP, EBRT and primary hormone therapy were 90%, 98%, and 89%, respectively.

CONCLUSION

The treatments provided by the participating institutions did not differ significantly from those set out in European and American guidelines, and short-term disease-specific survival rates for each treatment did not differ significantly from those of historical controls. Further investigation may facilitate the establishment of Japanese guidelines for the diagnosis and treatment of locally advanced prostate cancer.

KEYWORDS

locally advanced prostate cancer, radical prostatectomy, radiotherapy, hormone therapy

INTRODUCTION

The heterogeneity and wide spectrum of locally advanced prostate cancers make it difficult for urologists to determine what constitutes appropriate treatment for individual patients. Radical prostatectomy (RP), radiotherapy and hormone therapy are commonly used alone or combined. Numerous randomized clinical trials [1–9] and unrandomized studies [10–12] have been conducted on each therapeutic approach. Despite the many such clinical trials, the most appropriate therapeutic approach is still under debate in Japan, in terms of survival and quality of life (QoL). Moreover, neither the characteristics of patients with locally advanced prostate cancer nor the types of treatment they receive have been thoroughly investigated in Japan. Because it is necessary to understand these conditions before designing randomized clinical studies or establishing treatment guidelines, we investigated the characteristics of patients in Japan with locally advanced prostate cancer and who were treated at six institutions in eastern and north-eastern Japan from 1988 to 2000, and correlated these with their treatments and outcomes.

PATIENTS AND METHODS

The six Japanese academic institutions participating in this retrospective study were selected from independent institutions in eastern and north-eastern Japan. Patients with locally advanced prostate cancer who were treated at these institutions from 1988 to 2000 with RP, radiotherapy or hormone therapy as the primary method, and who also satisfied several other criteria, were included in the study.

The other criteria consisted of clinical diagnosis and staging in accordance with the TNM system [13], a PSA measurement at initial diagnosis, histopathological diagnosis by systematic TRUS-guided prostate biopsy, and availability of complete treatment records.

Information on 394 patients was compiled from the participating institutions; from this

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TABLE 1 The patients' characteristics by institution and by type of treatment

Variable	Median (range)		Biopsy tumour grade			Performance status		
	Age, years	PSA, ng/mL	Well	Moderate	Poor	0	1	2
Institution								
1	72.0 (58-87)	29.0 (2.0-435)	2	27	11	-	-	-
2	71.0 (57-89)	22.0 (4.7-100)	5	18	10	-	-	-
3	74.0 (51-90)	20.9 (0.6-571)	30	74	33	-	-	-
4	75.0 (55-88)	35.3 (2.3-754)	27	43	14	-	-	-
5	77.5 (64-84)	22.9 (58.3-141)	2	7	3	-	-	-
6	71 (58-92)	37.0 (6.0-538)	11	51	23	-	-	-
Total	73 (51-92)†	27.0 (0.6-754)†	77	220	94*	-	-	-
Type of treatment (n)								
RP (128)	69.0 (51-82)†	22.2 (2.4-434)*	21	77	30	119	9	0
Radiotherapy (87)	70.0 (56-84)	26.6 (2.0-371)	14	50	23	64	22	1
Hormone therapy (176)	78.0 (54-92)	33.7 (0.6-754)	42	93	41	133	41	2

*The Kruskal-Wallis and chi-square test were used to assess the correlation of patient characteristics between institutions; *P < 0.05, †< 0.01, ‡< 0.001.*

information, 391 were deemed to be eligible for the study. The clinicopathological characteristics of the patients, including age at diagnosis, performance status, pretreatment PSA level, and tumour grade, were investigated. Tumour grade was determined using the WHO system in each institution. To facilitate understanding of the patients' background and disease-specific survival (DSS), risk groups were defined by a combination of pretreatment serum PSA level and biopsy tumour grade. Patients with a PSA level of ≤ 10.0 ng/mL and a well or moderately differentiated tumour were classified as low risk; those with a PSA of 10.1-50 ng/mL and a well or moderately differentiated tumour as intermediate risk; and those with a PSA level of ≥ 50.1 ng/mL and/or a poorly differentiated tumour as high risk.

The type of treatment, including the application of neoadjuvant and/or adjuvant hormone therapy (N-, AHT) was investigated for the groups treated by RP or radiotherapy. The pathological stage after RP was evaluated according to the TNM system [13]. For the radiotherapy group, the type of radiation therapy, irradiation dose, and use of lymphadenectomy, and for the primary hormone therapy group, the type of hormone therapy, were investigated.

Because criteria for assessing comorbidity and QoL were not standardized across the participating institutions during the 1990s, comorbidity and QoL were not evaluated in this study. The length of follow-up, cause of death and time to death from prostate cancer or other causes were evaluated for each treatment group. Treatment outcome was assessed as DSS rates, using the Kaplan-Meier method, correlated with biopsy tumour grade, PSA level and risk group. The Mann-Whitney *U*-test, Kruskal-Wallis, chi-square and log-rank test were used for statistical analysis, as appropriate, with $P < 0.05$ considered to indicate statistical significance in all tests.

RESULTS

There was a statistically significant difference among institutions in patient age ($P = 0.001$), PSA distribution ($P = 0.011$) and tumour grade ($P = 0.050$), but no significant difference in the distribution of poorly differentiated tumours ($P = 0.564$; Table 1).

RP, radiotherapy and hormone therapy were used as primary treatments for 128, 87 and 176 patients, respectively (Table 2). Younger patients tended to receive a definitive treatment such as RP or radiotherapy as their primary treatment. The participating urologists tended to use RP for patients with lower PSA levels and/or good performance status. The PSA levels were highest in the primary hormone therapy group, lower in the radiotherapy group and lowest in the RP group ($P = 0.023$). There was no significant difference among the three therapeutic approaches in biopsy tumour grade ($P = 0.429$).

RP with no NHT (RP alone) was used in 60 of 128 patients, while NHT preceded RP in 68 (Table 2); NHT was used in those with higher PSA levels ($P = 0.012$). Immediate AHT after RP was used in 66 patients. In the 60 patients treated with RP alone, four of 14 with stage pT3a disease, seven of 12 with stage pT3b and 12 of 19 with stage pN1 received AHT; adjuvant radiotherapy was given to only two of the 12 patients with stage pT3b disease.

Of the 68 patients treated with RP after NHT, immediate AHT was used in 41; 10 of 24 with stage pT2, 10 of 16 with stage pT3a, four of seven with stage pT3b and 17 of 21 with pN1 disease received AHT after RP. Hormone therapy was the most common adjuvant therapy after RP.

In terms of clinical staging, 15 (25%) of the 60 patients treated with RP alone were diagnosed afterward as having stage pT2N0 disease (i.e. their disease was initially overstaged), and 19 (33%) as pN1 (i.e. their disease was initially understaged). Of the 68 patients treated with NHT and RP, despite higher PSA levels, the likelihood of the patient being diagnosed as having stage pT2N0 disease was higher, although this might not promise a longer PSA failure-free survival.

Table 2 also shows the characteristics of the 87 patients treated with radiotherapy. External beam radiotherapy (EBRT) of 70 Gy was used at institution 6, where three-dimensional conformal radiotherapy (3D-CRT) was also used. However, at the other

TABLE 2 The characteristics of patients treated with RP or radiotherapy

Median (range)	RP (128)			EBRT (75)			Brachytherapy (12)	Primary hormone therapy (176)
	RP alone (60)	+NHT (68)	P	Alone (19)	+ NHT (56)	P	+ EBRT (4)*	
Age, years	69.0 (55-79)	69.0 (51-83)	0.817	67.0 (52-82)	68.5 (56-79)	0.579	77.5 (64-84)	78.0 (54-92)
PSA, ng/mL	18.8 (3.6-170)	29.3 (2.4-434)	0.012	34.9 (7.9-337)	25.4 (2.0-371)	0.141	23.0 (8.3-141)	34.8 (0.6-754)
<10	16	12		1	12		3	29
10-50	36	39		12	28		6	83
>50	8	17	0.179	6	16	0.267	3	64
Biopsy tumour grade			0.319					
Well	11	10		3	9		2	42
Moderate	32	45		12	31		7	93
Poor	17	13		4	16	0.798	3	41
Risk group			0.704					
Low	19	17		0	9		2	21
Intermediate	20	25		10	21		4	70
High	21	26		9	26	0.147	6	85
Pathological stage			0.393					
pT2	15	24						
pT3	26	23						
pN1	19	21						
Lymph node dissection (20)								
pN0				0	17		0	
pN1				0	3		0	
Dose, Gy				60 (60-70)	66 (50-72)			
Method								
Standard EBRT				18	25		4	
3D-CRT				1	16		0	
Heavy particle RT				0	15		0	
NHT								
duration, months		4.8 (0.5-30)			4.0 (1-4)			
Method								
CAB		41			27			76
Castration including medical castration		16			20			85
Anti-androgen monotherapy		5			1			7
Oestrogen		7			8			8
Adjuvant therapy:								
+ hormone	23	41		8	40		0	
no hormone				11	16		12	
+ radiation	2	0						
no radiation	35	27						

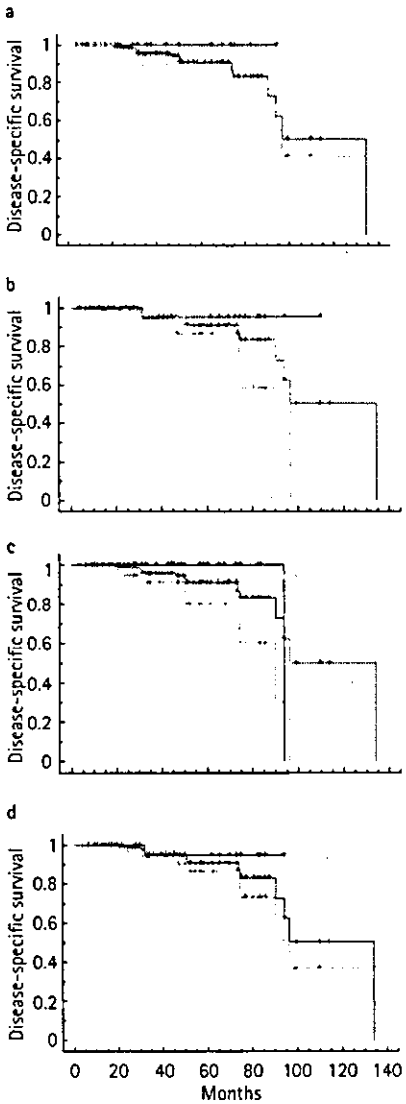
The Mann-Whitney U-test and chi-square test were used to evaluate the correlation of characteristics between treatment groups. This group was too small to calculate P values and includes all 12 patients. CAB, combined androgen blockade; RT, radiotherapy.

institutions radiation doses were generally lower. NHT was used in 56 of the 75 patients treated with EBRT with no brachytherapy, and AHT in 40 of the 56 and eight of the 19 who were treated with EBRT combined with NHT and EBRT alone, respectively. Therefore, some hormone therapies were concomitant in 64 of the 75 patients treated with EBRT with no brachytherapy (85%). Twenty of the 75 patients treated with EBRT and no brachytherapy had a lymphadenectomy; only 12 men received brachytherapy as primary therapy.

The characteristics of the 176 patients treated with primary hormone therapy are also shown in Table 2; for most of these patients, combined androgen blockade and medical/surgical castration were selected as the primary hormone therapy.

For treatment outcome, the median (range) follow-up for all eligible patients was 37 (1-137) months. The DSS rate was independently calculated for each treatment group. Of the RP group, 13 of the 128 patients died from prostate cancer; the DSS rates at 5 and 10 years for these patients were 90% and 49.5%, respectively (Fig. 1a-d). Patients with higher tumour grades tended to have the poorest DSS rates ($P = 0.316$; Fig. 1a). The P

FIG. 1. DSS curves for the 128 patients treated with RP, by tumour grade (a), PSA level (b), pathological stage (c) and (d) risk groups, as determined by the Kaplan-Meier method. In each plot the solid green line is for all patients; the red dotted line, light green dashed line and light red dashed/dotted line indicate respectively; (a) well, moderately and poorly differentiated tumour (P = 0.316); (b) PSA ≤ 10.0, 10.1–50.0 and >50 ng/mL (P = 0.102); (c) pT2N0, pT3N0 and pN1 (P = 0.002); (d) low-risk, intermediate-risk and high-risk groups (P = 0.184).



value indicated a modest difference in DSS rates between the groups stratified by initial PSA levels ($P = 0.102$; Fig. 1b). The group with a pretreatment PSA level of ≥50.1 ng/mL had the lowest DSS rate. The DSS rates by pathological stage at 5 years for patients at stages pT2, pT3 and pN1 with any pT were 100%, 93% and 79.5%, respectively ($P = 0.002$; Fig. 1c).

There was no statistically significant difference in the DSS rate among the risk groups ($P = 0.184$) but the DSS rate 7 years after surgery was 73% in the high-risk group (Fig. 1d), and lower than in the low and intermediate risk groups (96% and 90%, respectively).

FIG. 2. DSS curves for the 75 patients treated with EBRT or NHT and EBRT alone, by tumour grade (a), PSA level (b) and risk group (c), as determined by the Kaplan-Meier method. The key is the same as for Fig. 1. (a) P = 0.079, (b) P = 0.007, (c) P = 0.002, and (d) no P value generated.

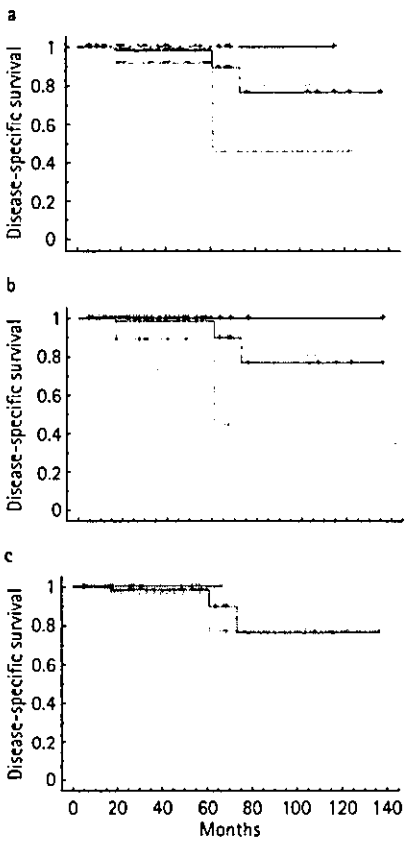


Fig. 2a–c shows the probability curves for the DSS of patients who received EBRT and NHT, and EBRT alone; three of the 75 patients died from prostate cancer during the follow-up; one had moderately differentiated and the other two poorly differentiated disease. The DSS rates at 5 and 10 years for these 75 patients were 98% and 76%, respectively. Patients with higher tumour grades had poor DSS rates ($P = 0.079$; Fig. 2a). However, paradoxically, the DSS rates were lower in patients with lower PSA levels ($P = 0.007$; Fig. 2b). Of patients with PSA levels of ≤10.0 ng/mL who died from prostate cancer, all had poorly differentiated carcinoma. The DSS rates at 5 years for both the low and intermediate risk groups was 100%; that for the high risk group was 96% (*P* not generated).

Of the 176 patients in the primary hormone therapy group, 11 died from prostate cancer; the DSS rate at 5 years for these patients was 89% (Fig. 3a–c). Patients with poorly differentiated tumours had the lowest DSS rate, although it was not statistically significant ($P = 0.309$) (Fig. 3a). There were no significant differences in DSS rates among the risk groups stratified by PSA levels ($P = 0.719$; Fig. 3b) nor among the risk groups ($P = 0.317$; Fig. 3c), but the rate at 5 years was 77% in the high risk group, which was lower than that in the low and intermediate risk groups (80% and 95%, respectively).

DISCUSSION

Therapeutic guidelines for locally advanced prostate cancer have been established in Europe [14] and the USA [15], but not yet in Japan. It remains controversial in Japan whether urologists should choose RP or radiotherapy with the aim of achieving potential cure, or use primary hormone therapy as palliative treatment. Although therapeutic guidelines have not yet been established in Japan, the therapeutic approaches to locally advanced prostate cancer assessed here were not significantly different from those set out in the European and American guidelines.

About half of all patients in Japan, Europe and the USA who were treated for locally advanced prostate cancer in the 1990s are reported to have been treated with NHT before RP [16,17]. Short-term NHT was also given to nearly half of the patients treated with RP in the present study. NHT was used to treat Japanese patients diagnosed to have more advanced disease; it can be assumed that the participating urologists preferred NHT during the present study period, supporting the hypothesis that NHT may

improve the complete cure rate. Most randomized clinical studies report that NHT significantly decreases the margin-positive rate [1,2], but randomized clinical trials have not shown that NHT improves the long-term survival rate after surgery [18,19]. Accordingly, there is serious doubt about whether NHT can be supported as a standard therapy.

In the present study, AHT after RP was most commonly used not only for treating stage pN1 disease, but also for stage pT3N0 disease, when adjuvant radiotherapy is often chosen in Europe and the USA for this stage [15,20]. Some studies report that AHT improves the progression-free survival rate [20,21] in pathological stage C patients. Conversely, Beyer *et al.* [22] stated that AHT for pathological locally advanced cancer or positive lymph nodes does not improve progression-free survival rates. Most of the previous studies were not randomized clinical studies and do not clearly identify the advantages of AHT for long-term survival. However, a recent randomized clinical trial showed that AHT for patients with positive lymph nodes improved overall survival, prostate cancer-specific survival, and clinical progression-free survival [3]. The frequent use of NHT and AHT in the present study may indicate that the participating urologists recognized the impact of staging error and determined that most clinical locally advanced prostate cancers might consist of systemic disease that would not be cured by RP alone.

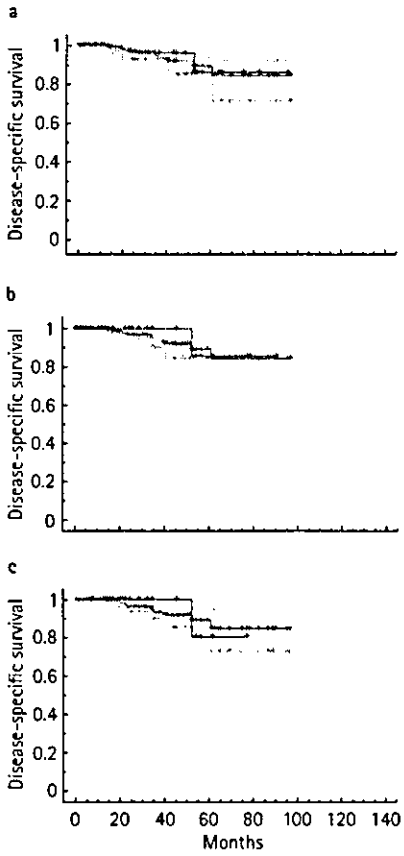
According to the guidelines [14,15] for therapeutic approaches to locally advanced prostate cancer, radiotherapy is the primary treatment option. In terms of using hormone therapy with radiotherapy, most recent reports found combined therapy to be beneficial. Although the mechanism of the interaction between radiotherapy and hormone therapy remains largely unknown, a possible additive or synergistic effect brought about by NHT has been reported [23]. The survival benefit provided by long-term AHT after radiation therapy has been shown by a randomized clinical trial [4] and meta-analysis [7] but prospective randomized trials should be undertaken to clarify which patients will not benefit at all, or benefit from short-term or only from long-term AHT [6]. In the present study, most patients received EBRT combined with hormone therapy; the results may indicate that combined therapy comprising radiotherapy and hormone therapy has been recognized as an effective treatment option by the participating urologists for the last decade.

The effectiveness of primary hormone therapy for many types of locally advanced prostate cancer was reported previously [24]. A recent report indicated that the benefits of hormone therapy alone are similar to those of other therapeutic approaches [25]. In the present study, primary hormone therapy alone tended to be used in relatively older patients or those with high PSA levels. We suggest that primary hormone therapy was chosen for relatively older patients with a higher risk of comorbidity, or for those with less chance of a complete cure.

Few randomized trials have been undertaken to evaluate the outcome of RP vs radiotherapy for locally advanced prostate cancer with no distant metastasis [5]. In the present study, the participating urologists tended to select RP more than radiation therapy for younger patients. However, most patients in this study had been diagnosed with stage pT3N0 or pN1 disease after RP and thus there may have been fewer candidates who should have been treated with RP. The choice of RP or radiotherapy for treating locally advanced prostate cancer will not become clear until well-controlled randomized studies provide sufficient evidence of long-term survival and improved QoL.

For treatment outcomes, the 5-year DSS rates after RP, radiotherapy and primary hormone therapy vary in the historical controls, at 83–92% [10,11], 50–100% [5,6], and 84–93% [9,24,25], respectively. The present results were not significantly different from these historical controls, although the characteristics of the patients were naturally biased among the reports. In the present RP group the patients with stage pT2 disease and even stage pT3 disease had longer DSS; the curves in the low- and intermediate-risk groups were satisfactory. However, the high-risk group comprising patients with a pretreatment PSA level of ≥50.1 ng/mL and/or a poorly differentiated tumour had the lowest long-term DSS, and thus probably affected the long-term DSS of the RP group. Thus, RP may also be an appropriate treatment for selected patients with either less advanced or low-grade tumours [11,12].

FIG. 3. DSS curves for the 176 patients treated with primary hormone therapy alone by tumour grade (a), PSA (b) and risk group (c), as determined by the Kaplan–Meier method. The key is the same as for Fig. 1. (a) P = 0.309, (b) P = 0.719, (c) P = 0.317.



Most of the patients who received radiotherapy concomitantly had EBRT and hormone therapy. This treatment strategy is similar to that set out in European and American guidelines but in the present study, lymph-node dissection and irradiation dose varied, so strictly the method of radiotherapy was heterogeneous. The present irradiation dose was generally lower than in other reports [4,6], although short-term DSS was not significantly different from that of the historical controls. However, this result does not necessarily suggest that even patients who are given a low irradiation dose will have a long DSS, because of the inherent bias of retrospective study, a short follow-up and the few patients. Studies involving increasing doses and combined therapy with NHT and AHT should be undertaken to clarify the effects of modern combined therapy. The

present results may also indicate the usefulness of primary hormone therapy in controlling locally advanced prostate cancer in relatively older patients with a higher risk of comorbidity, or patients with a lower possibility of complete cure.

Recent investigations indicate that different promoting factors, including genetic, epigenetic and environmental influences, might be responsible for ethnic variations in the progression of prostate cancer after its induction [26]. It has also been reported that there are large differences in prostate cancer incidence and plasma phyto-oestrogen concentration between Western and Japanese men [27]. Responses to therapy, e.g. hormone therapy, and the natural history after treatment might not be identical between ethnic groups which have considerable differences in prostate cancer incidence, plasma phyto-oestrogen concentration and racial composition, even if their standard of living, including health care, is similar. However, in the present study the primary treatments given to patients were not substantially different from those set out in European and American guidelines, and short-term DSS rates were not significantly different from those of the historical European and American controls. This might be significant, as the results were derived from an ethnic group in which prostate cancer incidence, dietary factors and racial composition differ considerably from those of Europeans and Americans. However, the results should be interpreted cautiously, because of inherent bias associated with this retrospective study. Therefore, it is necessary to undertake additional studies, including randomized controlled studies, to identify which therapeutic approach offers the best opportunity for survival and enhanced QoL for Japanese men. Further investigation may help to establish Japanese guidelines for the diagnosis and treatment of locally advanced prostate cancer.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **RP**, radical prostatectomy; **DSS**, disease-specific survival; **QoL**, quality of life; **NHT, AHT**, neoadjuvant, adjuvant hormone therapy; **EBRT**, external beam radiotherapy; **3D-CRT**, three-dimensional conformal radiotherapy.

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Blockade of Paclitaxel-Induced Thymidine Phosphorylase Expression Can Accelerate Apoptosis in Human Prostate Cancer Cells

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ABSTRACT

Recently, survival benefit by chemotherapy using paclitaxel (PTX) and the induction of thymidine phosphorylase (TP) by PTX have been reported in several solid tumors. On the other hand, TP confers antiapoptotic effect on tumor cells through inhibition of caspase-8 activation *in vitro*. On the basis of these previous observations, we hypothesized that (*a*) TP can be induced after PTX treatment in human prostate cancer (PC) and (*b*) blockade of PTX-induced TP expression can enhance the apoptotic processes in human PC cells. PTX was used to find TP expression in all eight hormone-refractory PC cases after chemotherapy; however, cleaved caspase-8 was not expressed after chemotherapy in the six hormone-refractory PC cases with strong TP expression. In PC cell lines (PC-3, DU 145, and LNCaP), TP expression after PTX treatment was clearly up-regulated in a dose-dependent manner. Cell viability of PC cell lines treated with PTX and TP antisense was significantly reduced in a time-dependent and dose-dependent manner compared with the PTX treatment alone. Likewise, apoptotic index of PC cells treated with PTX and TP antisense was significantly increased in comparison with PTX alone. After complete blockade of PTX-induced TP translation by TP antisense transfection, cleaved form of caspase-3 and poly(ADP-ribose) polymerase was increased, and this exaggeration of apoptosis also ran parallel with caspase-8 activation in a PTX dose-dependent manner. However, in PC cell lines treated with TP antisense alone, neither caspase-3 nor poly(ADP-ribose) polymerase was cleaved despite caspase-8 activation. These results indicate that PTX-induced TP up-regulation is associated with decreased caspase-8 activation. This study is the first report showing that blockade of PTX-induced TP expression could exaggerate the processing of apoptosis in PC cells treated with PTX. Our results provide preclinical evidence that TP could be a new molecular target for enhancing the potency of PTX-mediated apoptosis in PC cells.

INTRODUCTION

Thymidine phosphorylase (TP) is identical to platelet-derived endothelial cell growth factor and functions as chemotactic and angiogenic molecules (1). TP reversibly catalyzes the phosphorylation of thymidine to thymine and 2-deoxyribose-1-phosphate. TP also promotes tumor growth and confers resistance on apoptosis independent of angiogenesis, playing a key role in the invasiveness and metastasis of TP-expressing solid tumors (2). Recently, TP has been reported to inhibit Fas-induced caspase-8 activation, which in turn leads to the loss of mitochondrial membrane potential (3), and subsequently to prevention of the cytochrome *c* release, caspase-3 activation, and finally to inhibition of apoptosis (3). On the other hand, several lines of evidence show that TP is clearly induced in established human cancer cells by taxanes such as paclitaxel (PTX; ref. 4).

PTX, first isolated from the bark of the Western yew tree (*Taxus brevifolia*), is a complex diterpene that is distinct from other antimicrotubule agents in that it binds directly to polymerized tubulin, promoting microtubule assembly and stability (rather than instability) and preventing depolymerization (5). In clinical trials in which PTX is used in chemotherapy, PTX is generating excitement for the treatment of numerous types of tumors, including several refractory tumors such as ovarian carcinoma, myeloblastic leukemia, and hormone-refractory prostate cancer (HRPC; ref. 6–8).

Induction of apoptosis appears to be the main mechanism behind the antitumor effect of PTX (9, 10). Although several proteins involved in the PTX-mediated apoptosis have been identified, the molecular pathways underlying the apoptotic processes associated with PTX are not clearly defined (11–13). Understanding how PTX induces apoptosis is crucial to the elucidation of clinical relevance of chemotherapy using PTX. Furthermore, there is no definite link between TP expression and PTX-mediated apoptosis in cases of prostate cancer (PC). This relationship should be addressed to investigate the anticancer effect of PTX. In the present study, we examined TP expression in relation to apoptosis-related protein expression by an immunohistochemical analysis using HRPC samples before and after PTX-based chemotherapy, and human PC cell lines (PC-3, DU 145, and LNCaP) *in vitro* were used to attempt to elucidate whether TP influences PTX-mediated apoptosis or has cytoprotective function against PTX.

MATERIALS AND METHODS

Tissue Samples. Tissue samples from systematic sextant needle biopsy were collected in the same HRPC patients before and after PTX-based chemotherapy (14). From a total of 32 patients, eight cases (median age, 73.5; range, 54–80 years; clinical staging, $T_4N_0M_1$ in five and $T_4N_1M_1$ in three cases), whose biopsy samples before and after chemotherapy equally contained viable cancer cells within the same target areas, were recruited. Written form of informed consent was obtained from all patients. Tosoh II tPSA assay (Tosoh Medics, South San Francisco, CA) was used to measure serum levels of prostate-specific antigen (PSA) within a week just before and after chemotherapy.

Cell Culture. The human prostate cancer cell lines PC-3, DU145, and LNCaP were obtained from the American Type of Culture Collection (Manassas, VA) and incubated in F-12K and MEM-E supplemented with 10% fetal bovine serum and 2 mmol/L L-glutamine. The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air. Culture media were changed every 48 hours.

Immunohistochemistry. The tissue samples were fixed in 10% buffered formalin (pH 7.0) and processed for embedding in paraffin wax. The envision-peroxidase method (Dako, Carpinteria, CA) was used to perform immunohistochemical staining. Mouse monoclonal antibody against TP (1/100 dilution; Dako), rabbit polyclonal antibodies against cleaved caspase-8 and cleaved caspae-3 (1:1,000 dilution; Sigma, St. Louis, MO), and poly(ADP-ribose) polymerase (PARP) p85 fragment (1:100 dilution; Promega Corp., Madison, WI) were used in this study. The reaction products were visualized using diaminobenzidine (Dako). Normal mouse and rabbit IgG instead of monoclonal and polyclonal antibodies, respectively, served as a negative control. A pathologist not involved in the present study evaluated the immunostaining under an experimental blind condition. The immunohistochemical staining was graded on an arbitrary scale from 0 to 2+; 0 represents negative expression (0–20% positive cells), 1+ represents weakly positive expression (20–50% positive cells), and 2+ represents strongly positive expression (50–100% positive cells). The scale was determined according to the average rate of positive cells in ten random fields of all slides.

Morpholino Antisense Oligonucleotide Transfection. A specific morpholino antisense oligonucleotide (5′-GGTCATCAAGGCTGCCATCGCTCCG-3′)

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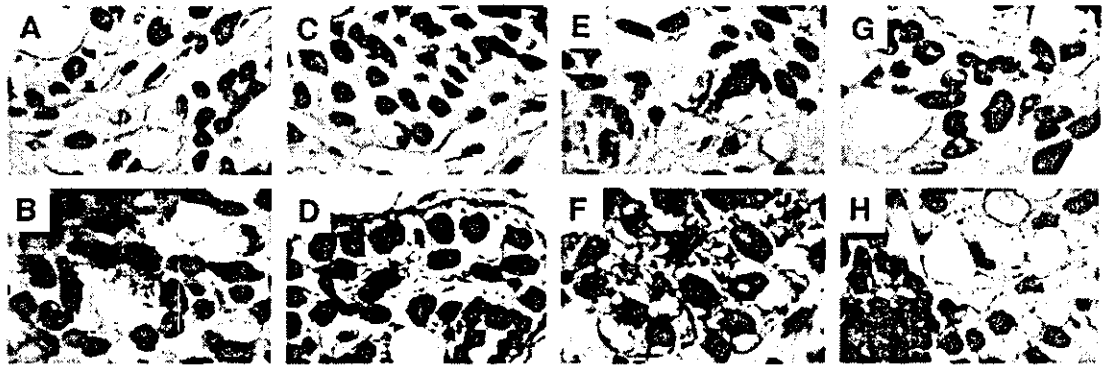

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Case #	Expression score								Serum PSA level (ng/ml)		
	TP		Cleaved caspase-8		Cleaved caspase-3		Cleaved PARP		Before	After	PSA reduction rate (%)
	Before	After	Before	After	Before	After	Before	After			
1	0	1+	0	1+	0	2+	0	2+	5.1	0.7	86.3
2	1+	1+	0	1+	0	2+	0	2+	13.2	1.5	88.6
3	1+	2+	0	0	0	2+	0	2+	3075.4	1481.4	51.8
4	1+	2+	0	0	0	2+	0	1+	580.0	67.0	88.4
5	1+	2+	0	0	0	2+	0	1+	42.6	2.9	93.2
6	2+	2+	0	0	0	2+	0	1+	69.0	2.7	96.1
7	2+	2+	0	0	0	1+	0	1+	683.0	41.6	93.9
8	2+	2+	0	0	0	1+	0	1+	17.5	1.5	91.4

Expression score
0: negative expression, 1+: weakly positive expression, 2+: strongly positive expression

Fig. 1. Representative immunostaining of TP (*A*, *B*), cleaved caspase-8 (*C*, *D*), cleaved caspase-3 (*E*, *F*), and cleaved PARP (*G*, *H*) in the prostate biopsy specimens having viable PC cells (×400) are shown. *A*, *C*, *E*, and *G*, before chemotherapy with PTX. PC cells in the tumor tissues that comprise small glandular structures are almost completely negative for TP (*A*), cleaved caspase-8 (*C*), cleaved caspase-3 (*E*), and cleaved PARP (*G*). All of these expressions were scored as 0. *B*, *D*, *F* and H, after chemotherapy with PTX. Small subsets of PC cells and stromal cells in the tumor tissues are weakly positive for TP (*B*) and cleaved caspase-8 (*D*) and are strongly positive for cleaved caspase-3 (*F*) and cleaved PARP (H). These expressions were scored as 1+, 1+, 2+ and 2+, respectively. *I*, summary of immunostaining data and PSA alteration before and after chemotherapy with PTX.

was designed complementary to the TP mRNA based on the entire cDNA sequence in the GenBank database (Genbank accession no. NM001953). Antisense containing 5 bp mismatch pairs (5′-GGTTATCAAAGCTACCGTCGCTTCG-3′) was used as a control sense oligonucleotide. For the oligonucleotide treatment of each cell line, a special delivery system following the manufacturer's protocol (Funakoshi, Tokyo, Japan) was used to transfect cells. The specificity (the degree of cross-hybridization with the entire sequence) was confirmed by Western blot analysis.

Growth Inhibition Assay. Cells treated with or without TP antisense were cultured for 24 hours in 6-well plates at a density of 5×10^5 cells/well in growth medium. Media were then changed to growth media containing PTX (1×10^{-9} to 1×10^{-7} mol/L). Cells were harvested with trypsin, and a hemocytometer was used to count them on days 2, 3, and 4. Experiments were done in quadruplicate for each time point.

MTT Assay. Cells were grown at 5×10^3 in the culture medium containing 100 μL of serum dispensed into 96-well microplates. After treatment with chemical agents, 10 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; 5 mg/ml; Sigma) was added to each well, and the plates were incubated at 37°C for 4 hours. A measurement wavelength of 450 nm and a reference wavelength of 655 nm was used to read absorbance on a microplate reader (Model 3550, Bio-Rad, Richmond, CA). IC_{50} was defined as the PTX concentration that inhibited cell growth by 50% compared with control cells, according to the average cell cycle length. IC_{50} values were calculated from a linear regression analysis of plotted values.

Evaluation of Apoptosis. The APOPercentage Apoptosis Assay kit (Biocolor Ltd. United Kingdom) was used to examine alteration in the apoptosis membrane. Briefly, cells were seeded at 5×10^3 in medium containing 100 μL of serum that was dispensed into 96-well microplates. After treatment with 1×10^{-9} PTX for 48 hours, the culture medium was replaced with fresh medium containing APOPercentage Dye Label. The APOP% Dye Release Reagent was added to each well to aid cell lyses and the release of the bound dye from the apoptotic cells. A microplate colorimeter was used to measure cell-bound dye recovered in solution. A reference wavelength of 655 nm was used to estimate the apoptotic index at a measurement wavelength of 595 nm.

Preparation of Cell Lysates. After drug treatment for 48 hours, cells were harvested with 0.02% trypsin, centrifuged, and the cell pellets immediately frozen at −80°C until use. Frozen tumor cells were homogenized in lysis buffer [1% Triton X-100; 20 mmol/L Tris-HCl (pH 7.6); 0.1% SDS; 1% sodium deoxycholate], lysates were centrifuged at 15,000 × *g* for 20 minutes at 4°C, and each supernatant was used in the immunoblotting analysis. Protein concentrations were determined by the Bradford method (15).

Immunoblotting. Samples were resolved by 11% SDS-PAGE. A Bio-Rad Transblot SD was used to electrophoretically transfer proteins to nitrocellulose membranes (Millipore Corp., Bedford, MA). The membranes were immersed in 5% skim milk in 0.02 mol/L Tris-HCl, 0.4 mol/L NaCl (pH 8.0), and 0.05% Tween 20 buffer (TTBS) for 1 hour and probed with monoclonal antibody against TP, caspase-8, and β-actin or polyclonal rabbit antibody against caspase-3, PARP, and cleaved PARP. Blots were then labeled with antimouse or antirabbit antibody conjugated with peroxidase; an enhanced chemiluminescence and Western blotting was used for visualization. The software package NIH was used to quantify Image Signal intensities. The protein expression level was depicted as the relative yield to the β-actin level.

Statistical Analysis. The difference between two groups was statistically analyzed by Mann-Whitney *U* test, or the Stat View V statistical package (SAS Institute Inc., Cary, NC) was used for Student's *t* test. The *P* value of <0.05 was regarded as statistically significant.

RESULTS

Immunostaining of TP and Apoptosis-Related Proteins in HRPC Specimens. PTX on the expression of TP and apoptosis-related cleaved form of caspase-8, capase-3, and PARP was used to

immunostain eight HRPC samples and assess the effect of chemotherapy. Typical immunostaining of TP, cleaved form of caspase-8, caspase-3, and PARP before and after chemotherapy was shown in Fig. 1. PTX in four HRPC cases (cases 1, 3, 4, and 5) was used to increase TP expression after chemotherapy, whereas the remaining four cases (cases 2, 6, 7, and 8) showed no difference in TP expression before and after chemotherapy. In all eight HRPC samples, cleaved forms of caspase-3 and PARP were not detectable before chemotherapy, whereas they were detectable after chemotherapy. In six HRPC samples (cases 3–8), cleaved form of caspase-8 expression was not detectable either before or after chemotherapy. On the other hand, the remaining two HRPC samples (cases 1 and 2) showed increased expression of cleaved caspase-8 after chemotherapy. In these 2 HRPC samples, cleaved form of caspase-3 and PARP expression was scored as 2+ (strongly positive expression) after chemotherapy.

Relationship between TP Expression and Serum PSA Levels before and after Chemotherapy with PTX A shown in Fig. 2, serum levels of PSA after chemotherapy were all reduced in the eight HRPC patients as compared with those before chemotherapy. Eight HRPC patients were divided into two groups based on the alteration of TP expression before and after chemotherapy with PTX; for group 1 (n = 4), TP expression was increased after chemotherapy, and for group 2 (n = 4), TP expression was not changed before and after chemotherapy. In group 1, PSA values before and after chemotherapy were 918.0 ± 731.4 and 387.6 ± 364.9, respectively, of which difference reached borderline significance (P = 0.07). Likewise, in group 2, PSA value before chemotherapy (203.0 ± 160.3) was significantly higher than that after chemotherapy (12.1 ± 9.8; P = 0.07). Furthermore, PSA reduction rate in group 2 was significantly higher (95.1 ± 0.53%) than that in group 1 (78.5 ± 9.2%; P = 0.02).

Effect of PTX on Cell Viability. After the treatment with PTX, cell growth of all three PC cell lines (PC-3, DU145, and LNCaP) was inhibited in a does-dependent and time-dependent manner. The PTX concentration required to completely inhibit cell growth was 1×10^{-8} mol/L in all 3 PC cell lines. If applied $>1 \times 10^{-8}$ mol/L concentration of PTX, cell counts in all of three PC cell lines were more likely to be reduced, indicating that the cell toxicity of PTX might be increased (data not shown). As shown in Fig. 3*A-C*, in the presence of TP antisense, the IC_{50} of PTX was significantly decreased ranging from 1.9×10^{-8} to 1.0×10^{-9} mol/L, 2.0×10^{-8} to 1.2×10^{-9} mol/L, and 9.0×10^{-9} to 1.0×10^{-9} mol/L for PC-3, DU145 and LNCaP, respectively. As shown in Fig. 3*D-F*, cell viability in the cells treated with 1×10^{-9} mol/L PTX alone or 1×10^{-9} mol/L PTX with TP antisense was more significantly reduced than that in the control cells ($P < 0.05$ and $P < 0.01$, respectively). However, in all 3 PC cell lines, there was no significant difference in cell viability between control and TP antisense treatment. Thus, stepwise decrease in cell viability was observed along with TP antisense treatment, PTX treatment, and combined treatment of TP antisense and PTX. In addition, the reduction of cell viability in these three PC cell lines treated with PTX + TP antisense was more time-dependent than in those cells treated with PTX alone.

Effect of PTX-Induced TP on Apoptosis. As shown in Fig. 4, we examined apoptosis using membrane alteration techniques on

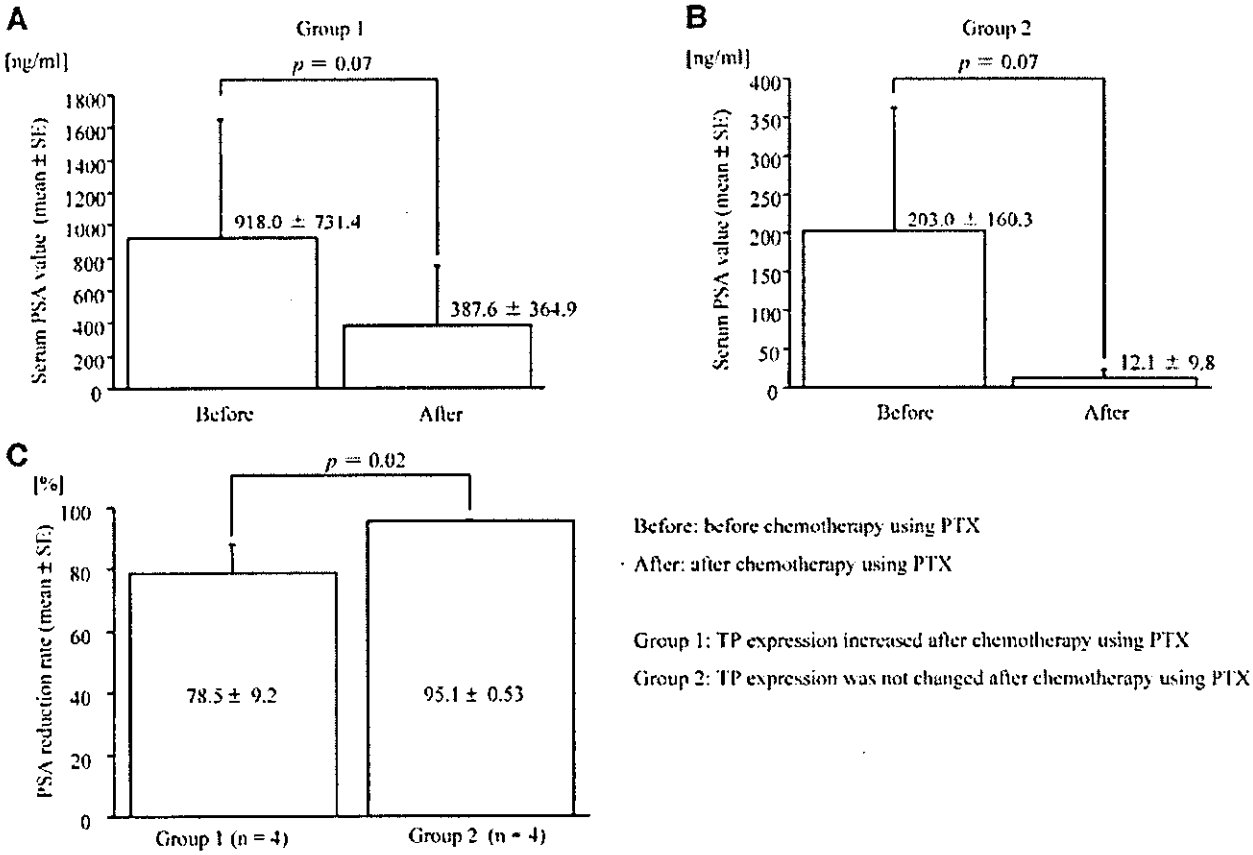

Fig. 2. Relationship of TP expression with PSA value and PSA reduction rate before and after chemotherapy with PTX in eight HRPC cases are shown. *A* and *B* show the alteration of serum PSA levels before and after chemotherapy with PTX. Group 1 includes four patients with increased TP expression after chemotherapy with PTX, whereas group 2 comprises the other four patients with TP expression being unchanged before and after chemotherapy. The difference in PSA reduction rate between Groups 1 and 2 is shown in *C*. Statistical significance was analyzed with Mann-Whitney *U* test. The values are expressed as mean ± SE.

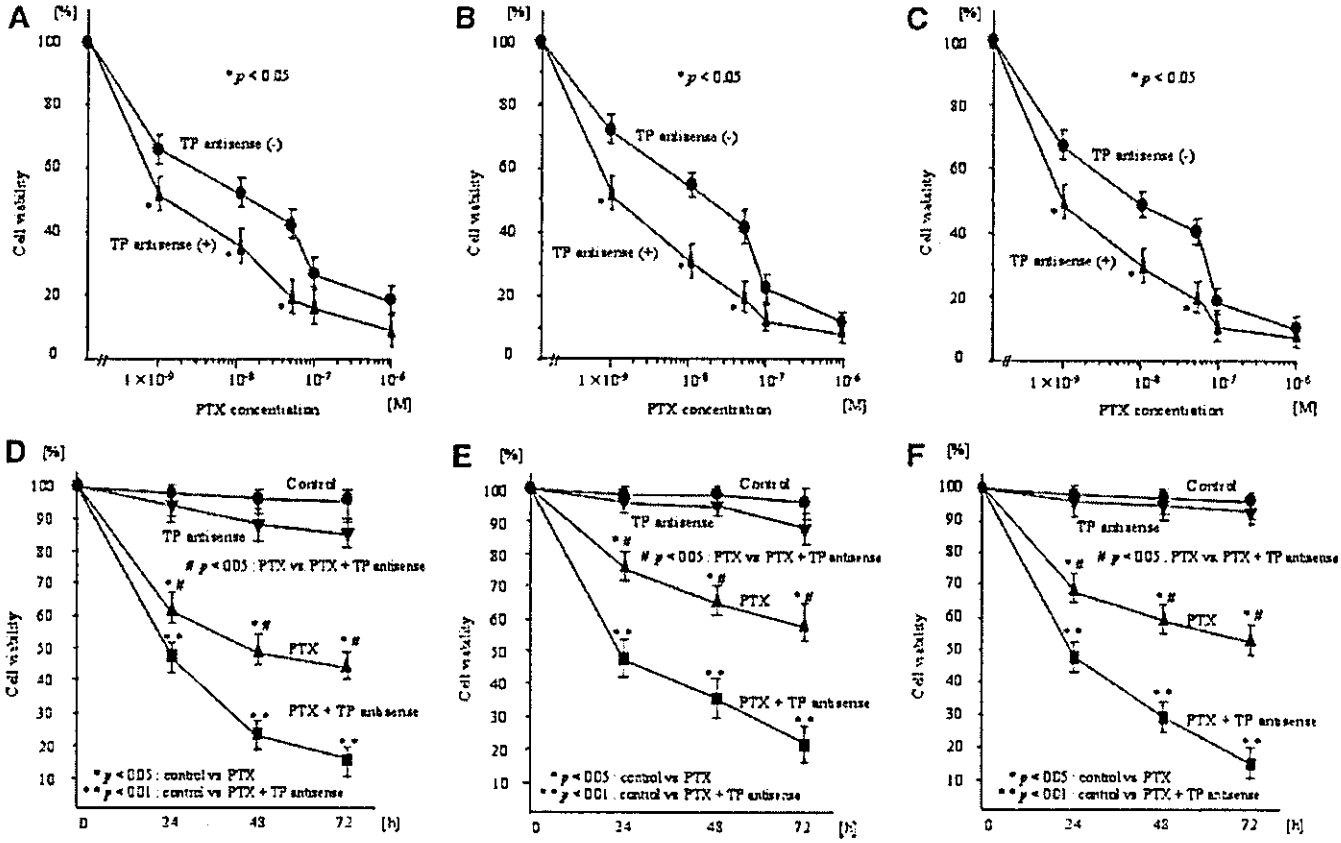

Fig. 3. Cell viability of PC cell lines treated with PTX and/or antisense TP is shown. *A*, *B*, and *C* show the dose-response curves of PC-3, DU 145, and LNCaP, respectively, and cells treated (▲) or untreated (●) with antisense oligonucleotide for TP mRNA (5′-GGTCATCAAGGCTGCCATCGCTCCG-3′) after exposure of PTX at five different concentrations (1×10^{-9}, 1×10^{-8}, 5×10^{-8}, 1×10^{-7}, and 1×10^{-6} mol/L) for 24 hours. Viable cells were measured by MTT assay and expressed as a percentage of the controls. *Asterisk* in Fig. 3*A-C* indicates the statistical difference in cell viability between TP antisense (−) and TP antisense (+) treated cell lines ($P < 0.05$). *D*, *E*, and *F* show the time-response curves of PC-3, DU145, and LNCaP cell lines, respectively. Cell viability of control nontreated PC cell lines (●), PC cell lines treated with TP antisense alone (▼), treated with 1×10^{-9} mol/L PTX alone (▲), and treated with a combination of both TP antisense and 1×10^{-9} mol/L PTX (■) for 24, 48, and 72 hours is shown. *Asterisks*, * and **, in Fig. 3*D-F* indicate the statistical difference in cell viability between control and PTX or between control and combination of PTX and TP antisense ($P < 0.05$and $P < 0.01$, respectively). # in Fig. 3*D-F* shows the statistical difference in cell viability between PTX and combination of PTX and TP antisense ($P < 0.05$). The values are expressed as mean ± SE at each point.

three PC cell lines treated with TP antisense alone or PTX of 1×10^{-9} mol/L alone, or a combination of both. Apoptotic index was standardized by that of control nontreated PC cell lines (no treatment of PTX and TP antisense) being as 1 and expressed as the arbitrary unit. In all PC cell lines, substantial stepwise increase of apoptotic index was observed along with PTX treatment alone (1.53 ± 0.12, 1.64 ± 0.14, and 1.66 ± 0.08 for PC-3, DU145 and LNCaP, respectively) and PTX treatment with TP antisense

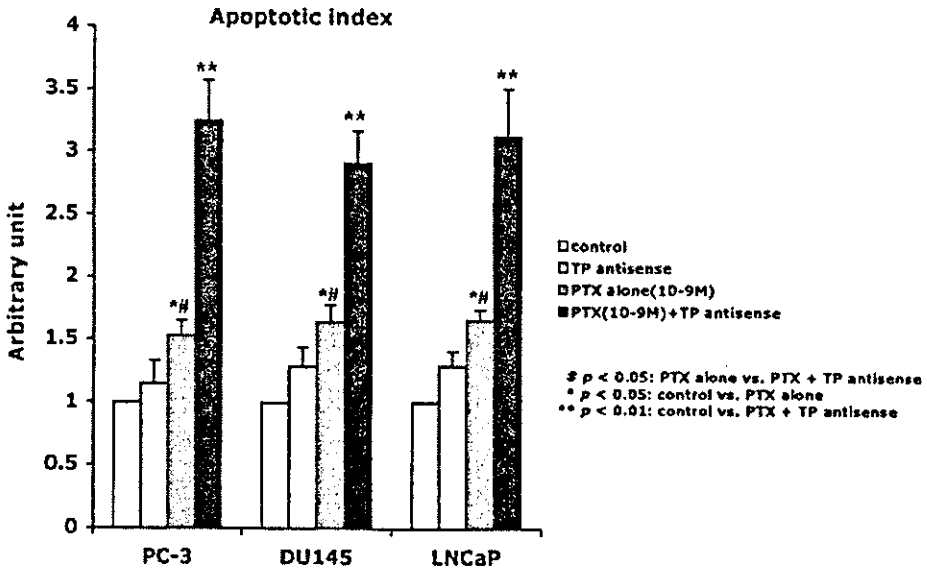

Fig. 4. Alteration of apoptotic index in PC cell lines with different treatment modalities is shown. Apoptotic indices of each group are expressed as its relative proportion to the untreated control (mean ± SE). Statistical significance was evaluated by student's *t* test. The difference in apoptotic index between PTX alone *versus* PTX + TP antisense, control *versus* PTX alone, and control *versus* PTX + TP antisense reached statistical significance ($P < 0.05$, $P < 0.05$, and $P < 0.01$, respectively).

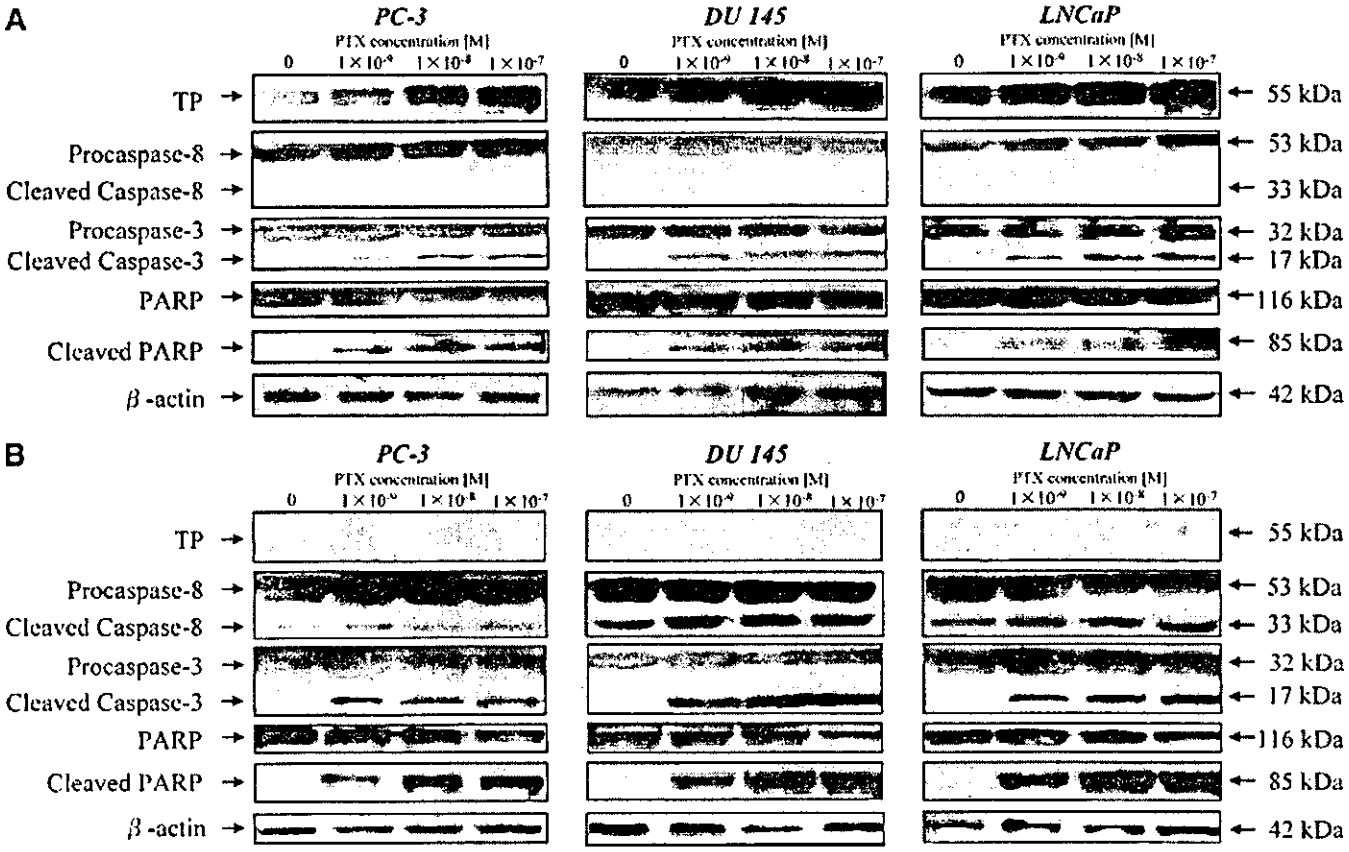

Fig. 5. Typical results of Western blotting of TP and apoptosis-related proteins in PC cell lines treated with different concentrations of PTX and/or TP antisense are shown. Protein levels of TP, procaspase-8, -3, cleaved caspase-8, -3, PARP, cleaved PARP and β-actin in PC-3, DU 145, and LNCaP cells untreated (*A*) or treated (*B*) with antisense oligonucleotide for TP mRNA. These cells were exposed to various concentrations of PTX (1×10^{-9} to 1×10^{-7} mol/L) for 48 hours. Different antibodies were used to apply total protein (100 μg) to Western blotting. The software package NIH Image was used to quantify signal intensities. Protein expression levels were depicted as their relative yield to the β-actin level.

(3.25 ± 0.32, 2.91 ± 0.26, and 3.13 ± 0.38 for PC-3, DU145 and LNCaP, respectively). Apoptotic index in PC cell lines treated with TP antisense alone was almost the same as that in the nontreated control PC cell lines.

Effect of PTX on TP Expression and Caspase-8, Caspase-3, and PARP Activation. Typical results of Western blotting were shown in Fig. 5. Western blot analysis showed that expression level of TP protein was increased dose-dependently in all of 3 PC cell lines treated with PTX alone. As shown in Fig. 6*A*, in PC-3, DU 145, and LNCaP cell lines treated with three different concentrations of PTX alone (1×10^{-9}, 1×10^{-8}, and 1×10^{-7} mol/L), the mean levels of TP expression were significantly higher than those in the control non-PTX-treated cells. In all of three PC cell lines treated with PTX alone, cleavage of caspase-3 and PARP, but not cleavage of caspase-8 was activated, whereas in the control non-PTX-treated cell lines none of the cleaved form of caspase-3, PARP, and caspase-8 was found. When TP expression was completely inhibited by TP antisense transfection, caspase-8 cleavage was activated in all of three PC cell lines. As shown in Fig. 6*B*, cleaved form of caspase-8 expression in PC-3, DU 145, and LNCaP cell lines treated with combination of PTX (1×10^{-9}, 1×10^{-8}, and 1×10^{-7} mol/L) and TP antisense transfection was significantly higher than cleaved form of caspase-8 expression in the control PC cells treated with TP antisense transfection alone. Moreover, the expression of cleaved form of caspase-3 and PARP in PC cell lines treated with a combination of PTX and TP antisense was significantly higher than the expression of those treated with PTX alone (Fig. 5).

DISCUSSION

In this study, all eight HRPC cases following chemotherapy with PTX expressed TP. Because up-regulation of cleaved form of caspase-3 and PARP was clearly observed, apoptotic process was exaggerated in these HRPC tissues. In six HPRC cases with strongly positive TP expression (cases 3–8) following chemotherapy, cleaved form of caspase-8 was not expressed either before or after chemotherapy with PTX, whereas in two HRPC cases with weakly positive TP expression (cases 1 and 2), cleaved caspase-8 was expressed, and apoptosis was strongly induced after chemotherapy. These observations might indicate that (*a*) chemotherapy using PTX-induced cell apoptosis and (*b*) cleaved caspase-8 expression was associated with down-regulation of TP expression and accelerated apoptosis. Considering the potential usefulness of PSA as a biological marker in PC patients (16), as shown in Fig. 2, the PSA reduction rate in patients with increased TP expression after chemotherapy was significantly lower than that observed in patients without increased TP expression. Thus, it might be clinically plausible that antitumor effect of PTX on tumor cells was diminished by simultaneous PTX-induced TP up-regulation. Another possibility is that the other chemotherapeutic agents other than PTX affect TP expression, because our series of HRPC patients underwent combined chemotherapy including not only PTX but also estramustine phosphate and carboplatin (14). However, our preliminary data revealed that either estramustine or carboplatin did not confer any substantial effect on the expression level of TP in PC-3 cell line (data not shown). On the basis of these findings, we hypothesized that blockade of PTX-induced TP might be essential for