Published OnlineFirst February 9, 2010; DOI:10.1158/0008-5472.CAN-09-2984

10. Chen CD, Welsbie DS, Tran C, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33–9.
11. Chen Y, Hughes-Fulford M. Prostaglandin E2 and the protein kinase A pathway mediate arachidonic acid induction of c-fos in human prostate cancer cells. Br J Cancer 2000;82:2000–6.
12. Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E2 induces hypoxia-inducible factor-1α stabilization and nuclear localization in a human prostate cancer cell line. J Biol Chem 2002;277:50081–6.
13. Wang X, Klein RD. Prostaglandin E2 induces vascular endothelial growth factor secretion in prostate cancer cells through EP2 receptor-mediated cAMP pathway. Mol Carcinog 2007;46:912–23.
14. Matsuoka T, Narumiya S. The roles of prostanoids in infection and sickness behaviors. J Infect Chemother 2008;14:270–8.
15. Yang L, Huang Y, Porta R, et al. Host and direct antitumor effects and profound reduction in tumor metastasis with selective EP4 receptor antagonism. Cancer Res 2006;66:9665–72.
16. Shah RB, Mehra R, Chinnaiyan AM, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004;64:9209–16.
17. Gleave ME, Hsieh JT, Wu HC, von Eschenbach AC, Chung LW. Serum prostate specific antigen levels in mice bearing human prostate LNCaP tumors are determined by tumor volume and endocrine and growth factors. Cancer Res 1992;52:1598–605.
18. Klein KA, Reiter RE, Redula J, et al. Progression of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice. Nat Med 1997;3:402–8.
19. Ellis WJ, Vessella RL, Buhler KR, et al. Characterization of a novel androgen-sensitive, prostate-specific antigen-producing prostatic carcinoma xenograft: LuCaP 23. Clin Cancer Res 1996;2:1039–48.
20. Marques RB, van Weerden WM, Erkens-Schulze S, et al. The human PC346 xenograft and cell line panel: a model system for prostate cancer progression. Eur Urol 2006;49:245–57.
21. Nagabhushan M, Miller CM, Pretlow TP, et al. CWR22: the first human prostate cancer xenograft with strongly androgen-dependent and relapsed strains both *in vivo* and in soft agar. Cancer Res 1996;56:3042–6.
22. Craft N, Chhor C, Tran C, et al. Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process. Cancer Res 1999; 59:5030–6.
23. Inoue T, Kobayashi T, Terada N, et al. Roles of androgen-dependent and -independent activation of signal transduction pathways for cell proliferation of prostate cancer cells. Expert Rev Endocrinol Metab 2007;2:689–704.
24. Wang Q, Li W, Zhang Y, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell 2009;138:245–56.
25. Nelson WG, De Marzo AM, Isaacs WB. Prostate Cancer. N Engl J Med 2003;349:366–81.
26. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001;1:34–45.
27. Taplin ME, Balk SP. Androgen receptor: a key molecule in the progression of prostate cancer to hormone independence. J Cell Biochem 2004;91:483–90.
28. Gregory CW, Johnson RT, Jr., Mohler JL, French FS, Wilson EM. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 2001;61: 2892–8.
29. Lee DK, Chang C. Endocrine mechanisms of disease: expression and degradation of androgen receptor: mechanism and clinical implication. J Clin Endocrinol Metab 2003;88:4043–54.
30. Kasbohm EA, Guo R, Yowell CW, et al. Androgen receptor activation by G(s) signaling in prostate cancer cells. J Biol Chem 2005;280: 11583–9.
31. Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in Apc δ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996;87:803–9.
32. Hull MA, Ko SC, Hawcroft G. Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer? Mol Cancer Ther 2004;3:1031–9.
33. Platz EA, De Marzo AM. Epidemiology of inflammation and prostate cancer. J Urol 2004;171:S36–40.
34. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer 2007;7:256–69.
35. Mahmud S, Franco E, Aprikian A. Prostate cancer and use of nonsteroidal anti-inflammatory drugs: systematic review and meta-analysis. Br J Cancer 2004;90:93–9.
36. Gupta S, Adhami VM, Subbarayan M, et al. Suppression of prostate carcinogenesis by dietary supplementation of celecoxib in transgenic adenocarcinoma of the mouse prostate model. Cancer Res 2004; 64:3334–43.
37. Singer EA, Palapattu GS, van Wijngaarden E. Prostate-specific antigen levels in relation to consumption of nonsteroidal anti-inflammatory drugs and acetaminophen: results from the 2001-2002 National Health and Nutrition Examination Survey. Cancer 2008;113:2053–7.
38. Smith MR, Manola J, Kaufman DS, Oh WK, Bubley GJ, Kantoff PW. Celecoxib versus placebo for men with prostate cancer and a rising serum prostate-specific antigen after radical prostatectomy and/or radiation therapy. J Clin Oncol 2006;24:2723–8.
39. Lieberman R. Chemoprevention of prostate cancer: current status and future directions. Cancer Metastasis Rev 2002;21:297–309.
40. Csiki I, Morrow JD, Sandler A, et al. Targeting cyclooxygenase-2 in recurrent non-small cell lung cancer: a phase II trial of celecoxib and docetaxel. Clin Cancer Res 2005;11:6634–40.
41. Ray WA, Stein CM, Daugherty JR, Hall K, Arbogast PG, Griffin MR. COX-2 selective non-steroidal anti-inflammatory drugs and risk of serious coronary heart disease. Lancet 2002;360:1071–3.
42. Sugimoto Y, Narumiya S. Prostaglandin E receptors. J Biol Chem 2007;282:11613–7.
43. Sonoshita M, Takaku K, Sasaki N, et al. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Δ716) knockout mice. Nat Med 2001;7:1048–51.
44. Jain S, Chakraborty G, Raja R, Kale S, Kundu GC. Prostaglandin E2 regulates tumor angiogenesis in prostate cancer. Cancer Res 2008; 68:7750–9.
45. Iljin K, Wolf M, Edgren H, et al. TMPRSS2 fusions with oncogenic ETS factors in prostate cancer involve unbalanced genomic rearrangements and are associated with HDAC1 and epigenetic reprogramming. Cancer Res 2006;66:10242–6.
46. Chell S, Kaidi A, Williams AC, Paraskeva C. Mediators of PGE2 synthesis and signalling downstream of COX-2 represent potential targets for the prevention/treatment of colorectal cancer. Biochim Biophys Acta 2006;1766:104–19.
47. Mutoh M, Watanabe K, Kitamura T, et al. Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis. Cancer Res 2002;62:28–32.
48. Chell SD, Witherden IR, Dobson RR, et al. Increased EP4 receptor expression in colorectal cancer progression promotes cell growth and anchorage independence. Cancer Res 2006;66:3106–13.

Published OnlineFirst February 9, 2010; DOI:10.1158/0008-5472.CAN-09-2984

Cancer Research

Correction: Online Publication Dates for *Cancer Research* April 15, 2010 Articles

The following articles in the April 15, 2010 issue of *Cancer Research* were published with an online publication date of April 6, 2010 listed, but were actually published online on April 13, 2010:

Garmy-Susini B, Avraamides CJ, Schmid MC, Foubert P, Ellies LG, Barnes L, Feral C, Papayannopoulou T, Lowy A, Blair SL, Cheresh D, Ginsberg M, Varner JA. Integrin $\alpha4\beta1$ signaling is required for lymphangiogenesis and tumor metastasis. Cancer Res 2010;70:3042–51. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3761.

Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, Martin F, Apetoh L, Rébé C, Ghiringhelli F. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 2010;70:3052–61. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3690.

Nagasaka T, Rhees J, Kloor M, Gebert J, Naomoto Y, Boland CR, Goel A. Somatic hypermethylation of *MSH2* is a frequent event in Lynch syndrome colorectal cancers. Cancer Res 2010;70:3098–108. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3290.

He X, Ota T, Liu P, Su C, Chien J, Shridhar V. Downregulation of HtrA1 promotes resistance to anoikis and peritoneal dissemination of ovarian cancer cells. Cancer Res 2010;70:3109–18. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3557.

Fiorentino M, Judson G, Penney K, Flavin R, Stark J, Fiore C, Fall K, Martin N, Ma J, Sinnott J, Giovannucci E, Stampfer M, Sesso HD, Kantoff PW, Finn S, Loda M, Mucci L. Immunohistochemical expression of BRCA1 and lethal prostate cancer. Cancer Res 2010;70:3136–9. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4100.

Veronese A, Lupini L, Consiglio J, Visone R, Ferracin M, Fornari F, Zanesi N, Alder H, D'Elia G, Gramantieri L, Bolondi L, Lanza G, Querzoli P, Angioni A, Croce CM, Negrini M. Oncogenic role of *miR-483-3p* at the *IGF2/483* locus. Cancer Res 2010;70:3140–9. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4456.

Lu W, Zhang G, Zhang R, Flores LG II, Huang Q, Gelovani JG, Li C. Tumor site–specific silencing of *NF-κB p65* by targeted hollow gold nanosphere–mediated photothermal transfection. Cancer Res 2010;70:3177–88. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3379.

Geng H, Rademacher BL, Pittsenbarger J, Huang C-Y, Harvey CT, Lafortune MC, Myrthue A, Garzotto M, Nelson PS, Beer TM, Qian DZ. ID1 enhances docetaxel cytotoxicity in prostate cancer cells through inhibition of p21. Cancer Res 2010;70:3239–48. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3186.

Yoo BK, Chen D, Su Z-z, Gredler R, Yoo J, Shah K, Fisher PB, Sarkar D. Molecular mechanism of chemoresistance by astrocyte elevated gene-1. Cancer Res 2010;70:3249–58. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4009.

Lu ZH, Shvartsman MB, Lee AY, Shao JM, Murray MM, Kladney RD, Fan D, Krajewski S, Chiang GG, Mills GB, Arbeit JM. Mammalian target of rapamycin activator RHEB is frequently overexpressed in human carcinomas and is critical and sufficient for skin epithelial carcinogenesis. Cancer Res 2010;70:3287–98. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3467.

Hattermann K, Held-Feindt J, Lucius R, Müerköster SS, Penfold MET, Schall TJ, Mentlein R. The chemokine receptor CXCR7 is highly expressed in human glioma cells and mediates antiapoptotic effects. Cancer Res 2010;70:3299–308. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3642.

Nadiminty N, Lou W, Sun M, Chen J, Yue J, Kung H-J, Evans CP, Zhou Q, Gao AC. Aberrant activation of the androgen receptor by NF-κB2/p52 in prostate cancer cells. Cancer Res 2010;70:3309–19. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3703.

Acu ID, Liu T, Suino-Powell K, Mooney SM, D'Assoro AB, Rowland N, Muotri AR, Correa RG, Niu Y, Kumar R, Salisbury JL. Coordination of centrosome homeostasis and DNA repair is intact in MCF-7 and disrupted in MDA-MB 231 breast cancer cells. Cancer Res 2010;70:3320–8. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3800.

McFarlane C, Kelvin AA, de la Vega M, Govender U, Scott CJ, Burrows JF, Johnston JA. The deubiquitinating enzyme USP17 is highly expressed in tumor biopsies, is cell cycle regulated, and is required for G_1-S progression. Cancer Res 2010;70:3329–39. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4152.

Dudka AA, Sweet SMM, Heath JK. Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification. Cancer Res 2010;70:3391–401. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3033.

Cho SY, Xu M, Roboz J, Lu M, Mascarenhas J, Hoffman R. The effect of CXCL12 processing on $CD34^+$ cell migration in myeloproliferative neoplasms. Cancer Res 2010;70:3402–10. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3977.

Published OnlineFirst 05/11/2010.

doi: 10.1158/0008-5472.CAN-10-1347

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010;40(6)588–592
doi:10.1093/jjco/hyq008
Advance Access Publication 18 March 2010

Trends of the Primary Therapy for Patients with Prostate Cancer in Nara Uro-oncological Research Group (NUORG): A Comparison Between the CaPSURE Data and the NUORG Data

Nobumichi Tanaka*, Kiyohide Fujimoto, Akihide Hirayama, Tatsuo Yoneda, Katsunori Yoshida and Yoshihiko Hirao

Department of Urology, Nara Medical University, Nara, Japan

*For reprints and all correspondence: Nobumichi Tanaka, Department of Urology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan. E-mail: sendo@naramed-u.ac.jp

Received September 8, 2009; accepted January 13, 2010

Objective: We assessed the variations in stage, prostate specific antigen at diagnosis, Gleason score, risk classification and primary therapy in Japanese prostate cancer patients, and compared with those of the US patients.

Methods: Between 2004 and 2006, the distribution of primary therapy and clinical characteristics of 2303 newly diagnosed patients at Nara Medical University and its 23 affiliated hospitals were assessed to compare with those of the Cancer of the Prostate Strategic Urological Research Endeavor data and to clarify the differences in data between the USA and Japan.

Results: The proportions of clinical T stage of 3–4, prostate specific antigen at diagnosis >20 ng/ml, Gleason score of 8–10 and high-risk group were greater in our study than those of the Cancer of the Prostate Strategic Urological Research Endeavor data (T3–4, 26.2 vs. 3.5–11.8%; prostate-specific antigen, 34.1 vs. 8.1–27.0%; Gleason score, 29.3 vs. 9.7–12.1%). Regarding the primary treatments, 51% of patients received primary androgen deprivation therapy, 30% underwent radical prostatectomy, 14% received radiation therapy and 2% had watchful waiting in our study, while the corresponding figures in the Cancer of the Prostate Strategic Urological Research Endeavor data were: radical prostatectomy, 44%; radiation therapy, 23%; primary androgen deprivation therapy 20% and watchful waiting 10%.

Conclusions: The Japanese prostate cancer patients still have higher prostate-specific antigen at diagnosis, higher Gleason score and higher clinical stage than the US patients. The trends of primary therapy for prostate cancer were different from those in the USA. The higher rate of primary androgen deprivation therapy is characteristic for the Japanese patients.

Key words: primary therapy – prostate cancer – staging – risk classification – Gleason score

INTRODUCTION

Since the introduction of prostate-specific antigen (PSA) in cancer screening, most patients with prostate cancer has demonstrated lower Gleason score, lower PSA at diagnosis and lower stage when compared with those before 1990s in the USA (1). Men of 50 years or older were highly exposed to a PSA test in the USA (2). On the other hand, the PSA-exposure rate in the Japanese men is unfortunately still lower than that of those in the USA (3), and the stage migration following the beginning of PSA screening has not been accomplished in Japan.

The CaPSURE data showed that, between 2000 and 2002, the distributions of low-, intermediate-, and high-risk patients were 46.8, 37.2 and 16.0%, respectively. Regarding the primary therapy, approximately 40% of the patients

underwent radical prostatectomy (RP) and 20% patients received primary androgen deprivation therapy (PADT) according to the report of Veterans Affairs (VA) health care system (4). On the other hand, the breakdown of primary therapies of the Japanese patients was different from that of the Americans. In 2000, 57% of the Japanese patients received PADT and 28% received RP in the population where more than 33% of the patients showed clinical T3 or T4 and 48% showed PSA of 20 ng/ml or greater at diagnosis (5). The distribution of risk classification and the breakdown of the primary therapy in Japan are supposed to be different from those in the USA.

In this study, we surveyed patients newly diagnosed as having prostate cancer in our institution and the affiliated hospitals to assess their clinicopathologic characteristics and primary therapy comparing with the CaPSURE data in the USA.

PATIENTS AND METHODS

A total of 2371 patients who were newly diagnosed as having prostate cancer at Nara Medical University (NMU) hospital and its 23 affiliated hospitals between January 2004 and December 2006 were enrolled in this study. Sixty-eight patients were excluded from this study due to incomplete information at diagnosis. Finally, 2303 patients were evaluated. The clinical T stage, PSA at diagnosis, Gleason score, age and primary therapy were investigated in all patients. The patients were divided into low-, intermediate- and high-risk groups according to the risk classification of the CaPSURE data (2003) (1). For instance, low-risk patients had PSA of 10 ng/ml or less, biopsy Gleason score of 6 or less and clinical stage (1997 TNM system) T1 or T2a. Intermediate-risk patients had a PSA of 10.01–20 ng/ml, Gleason score of 7 and clinical stage T2b. High-risk patients had PSA >20 ng/ml, Gleason score of 8 or greater and clinical stage T3 or T4. These classifications were based on the criteria of D'Amico et al. (6), with the exception of the TNM staging system (D'Amico et al. adopted the 1992 TNM classification).

The distribution of primary therapy and the clinical characteristics of this study were compared with those of the CaPSURE data as an example to clarify the differences in data between the USA and Japan. The statistical analysis was not available in comparison of clinical characteristics because the number of patients in each period (1989–1992, 1993–1995, 1996–1999 and 2000–2002) of the CaPSURE data was not described (1).

The distribution of primary therapies based on the clinical parameters and risk classification of the cohorts in this study was compared with those of the CaPSURE data (4) (2003) in the USA.

The primary therapies of the low-risk group in which available modalities were most varied were compared with those of the CaPSURE data of between 2004 and 2006 (7). In this comparison, the risk classification was based on the criteria of D'Amico et al. (6) which was also used in the CaPSURE data (2007) (7).

To examine the differences in categorical parameters, χ^2 test was performed. All P-values <0.05 were considered as statistically significant.

The institutional reviewer board approved this retrospective study, and obtaining informed consent from the patients was exempted in the respect of the aim and methods of this study.

RESULTS

Of all 2303 patients, 420 patients were treated at NMU hospital and 1883 were treated at 23 affiliated hospitals. The mean age and PSA at diagnosis of all patients were 71.9 years (median 72; range 39–96) and 140.0 ng/ml (median 12.3, range, 0.4–16 920), respectively. The demography of 2303 patients is shown separately in the university hospital and the affiliate hospitals in Table 1. In NMU hospital, the patient's age was younger, PSA at diagnosis was lower, Gleason score was lower and clinical T stage was lower than those in the affiliated hospitals ($P < 0.001$).

The patients' demographics at diagnosis such as PSA value, Gleason score, clinical T stage and risk classification in this study were also different from those of the CaPSURE data (1) (Fig. 1). In our series, 41.8% of the patients showed PSA values of 10 or less, 33.3% of them had T1 stage and 39.3% of them showed Gleason score of 6 or less. Of all patients, 503 patients (21.8%) were considered as the low-risk group, 650 patients (28.3%) as the intermediate-risk group and 1150 patients (49.9%) as the high-risk group. On the other hand, the CaPSURE data showed that 47.9% of the patients between 1989 and 1992, 57.5% between 1993 and 1995, 66.0% between 1996 and 1999, and 76.1% between 2000 and 2002 showed PSA values of 10 or less. Regarding clinical T stage and Gleason score, 26.2 and 29.3% of the patients showed T3–4 and Gleason score of 8–10 in this study, while 3.5 and 9.8% of the patients showed T3–4 and Gleason score of 8–10 in the CaPSURE data of between 2000 and 2002. Our patients diagnosed between 2004 and 2006 had higher PSA value, higher Gleason score, higher clinical T stage and higher risk classification than the patients of the CaPSURE data diagnosed between 1989 and 2002.

In our series of patients, 51% received PADT, whereas 20% received PADT in the CaPSURE data (4). The proportion of RP was 30% for our patients, whereas it was 44% in the CaPSURE data. Regarding the external beam radiation therapy (EBRT), the proportions in our series and the CaPSURE data were 8 and 16%, respectively. The proportion of brachytherapy was 6% for our patients and 7% for CaPSURE patients. The proportion of WW in our series was 2% and smaller than that of the CaPSURE data (10%) ($P < 0.001$) (Fig. 2).

Table 1. Demographic characteristic of 2303 patients

	Overall [$n = 2303$ (%)]	University hospital [$n = 420$ (%)]	Affiliated hospital [$n = 1883$ (%)]	P value (χ^2 test)
Age				
<60	154 (6.7)	52 (12.4)	102 (5.4)	0.001>
60–69	684 (29.7)	130 (31.0)	554 (29.4)	
70–79	1117 (48.5)	199 (47.3)	918 (48.8)	
≥80	348 (15.1)	39 (9.3)	309 (16.4)	
PSA at diagnosis				
<4.0	37 (1.6)	(92.1)	28 (1.5)	0.001>
4.0–10.0	926 (40.2)	216 (51.4)	710 (37.7)	
10.1–20	554 (24.1)	98 (23.3)	456 (24.2)	
>20	785 (34.1)	97 (23.1)	689 (36.6)	
Gleason score				
2–4	72 (3.1)	0 (0.0)	72 (3.8)	0.001>
5–6	834 (36.2)	146 (34.8)	688 (36.5)	
7	722 (31.4)	175 (41.6)	547 (29.0)	
8–10	675 (29.3)	99 (23.6)	576 (30.6)	
Clinical T stage				
T1	766 (33.3)	183 (43.6)	583 (31.0)	0.001>
T2	933 (40.5)	134 (31.9)	799 (42.4)	
T3	489 (21.2)	95 (22.6)	394 (20.9)	
T4	115 (5.0)	8 (1.9)	107 (5.7)	

PSA, prostate-specific antigen.

Of all patients, 503 were considered the low-risk group according to the CaPSURE data (2003) (1). Of these 503 patients, 468 were classified into the low-risk group according to the D'Amico's classification (6). The primary therapy for the low-risk patients showed a significant higher proportion of PADT and a significant lower proportion of RP in our series than in the CaPSURE data (2007) ($P < 0.001$) (Fig. 3).

DISCUSSION

Cooperberg et al. (1) demonstrated the time trends in risk stratification for prostate cancer using the CaPSURE data in 2003. From 1989 to 2002, the clinical T stage, Gleason score and PSA at diagnosis of newly diagnosed patients showed dramatic changes. Earlier time of diagnosis and the numbers of patients showing lower PSA, lower Gleason score and lower clinical T stage increased. Consequently, the high-risk patients significantly decreased, whereas the low-risk patients significantly increased. From 2000 to 2002, 46.8% patients were classified as the low-risk group and 16.0% were done as the high-risk group, whereas from 1989 to 1992, 29.5 and 36.5% patients were classified as the low- and high-risk groups, respectively.

On the other hand, the newly diagnosed Japanese patients in our study still had higher PSA value, higher Gleason score, advanced clinical T stage, and worse risk classification as compared with those of the CaPSURE data. The conceivable reason of this discrepancy between the Japanese and American patients is the difference of exposure rate of PSA screening. Approximately 75% of the American men aged 50 years or more measured their own PSA value (2) while the PSA-exposure rate in the Japanese men is currently still low (3). Accordingly, this study possesses a potential limitation in comparing the primary therapy between the two countries having different PSA-exposure rates. Our data demonstrated the difference in the proportion of PSA value, Gleason score and clinical T stage when compared with the CaPSURE data. In other words, the Japanese urologists even now treat patients who have similar characteristics in USA a decade ago.

Regarding the primary therapy, a preferable trend to choose PADT was noticed in the Japanese patients (5,8–10). In 2000, approximately 57% of 4529 patients received PADT in Japan (5). Of the patients with T1c-T3N0M0, 1226 patients (45.9%) chose PADT (5). In our present study, 42% (796 of 1887) of the patients with T1c-T3N0M0 chose PADT. Furthermore, 27% of the low-risk patients in our present survey chose PADT. The proportion of PADT was high in our low-risk group patients as well as in the Japanese patients with localized and locally advanced prostate cancer. On the other hand, only 7% of the low-risk group patients received PADT in the CaPSURE data (7).

In contrast to PADT, the proportions of RP and RT in the CaPSURE data were higher than those in our present study (RP, 44 vs. 30%; RT, 23 vs. 14%). Furthermore, the proportion of WW in our present study was lower than that in the CaPSURE data (2 vs. 10%, respectively). In NMU hospital, 3-DCRT, IMRT and seed implant are available. On the other hand, 3-DCRT is available in several affiliated hospitals but not in most of the affiliated hospitals. In NMU hospital, 44% of the patients received RT (e.g. IMRT, 3-DCRT or seed implant) and 24% received PADT while in the affiliated hospitals 7% received RT and 57% received PADT (data not shown). The proportion of PADT in NMU hospital was not different from that of the CaPSURE data (24 vs. 20%, respectively) (data not shown). The main reason for this trend in NMU hospital is that NMU hospital is the only institute to provide three modalities (IMRT, seed implant and RP) for our patients. In NMU hospital, the patients with newly diagnosed prostate cancer are recommended several therapeutic options and there are many referred patients who intend to receive IMRT or seed implant.

In the affiliated hospitals, the physicians are apt to provide PADT if the patients hesitate to receive RP. If EBRT is available, the physicians can provide EBRT instead of RP. In addition, most patients are likely to receive primary therapy in the same hospital where their prostate cancers are

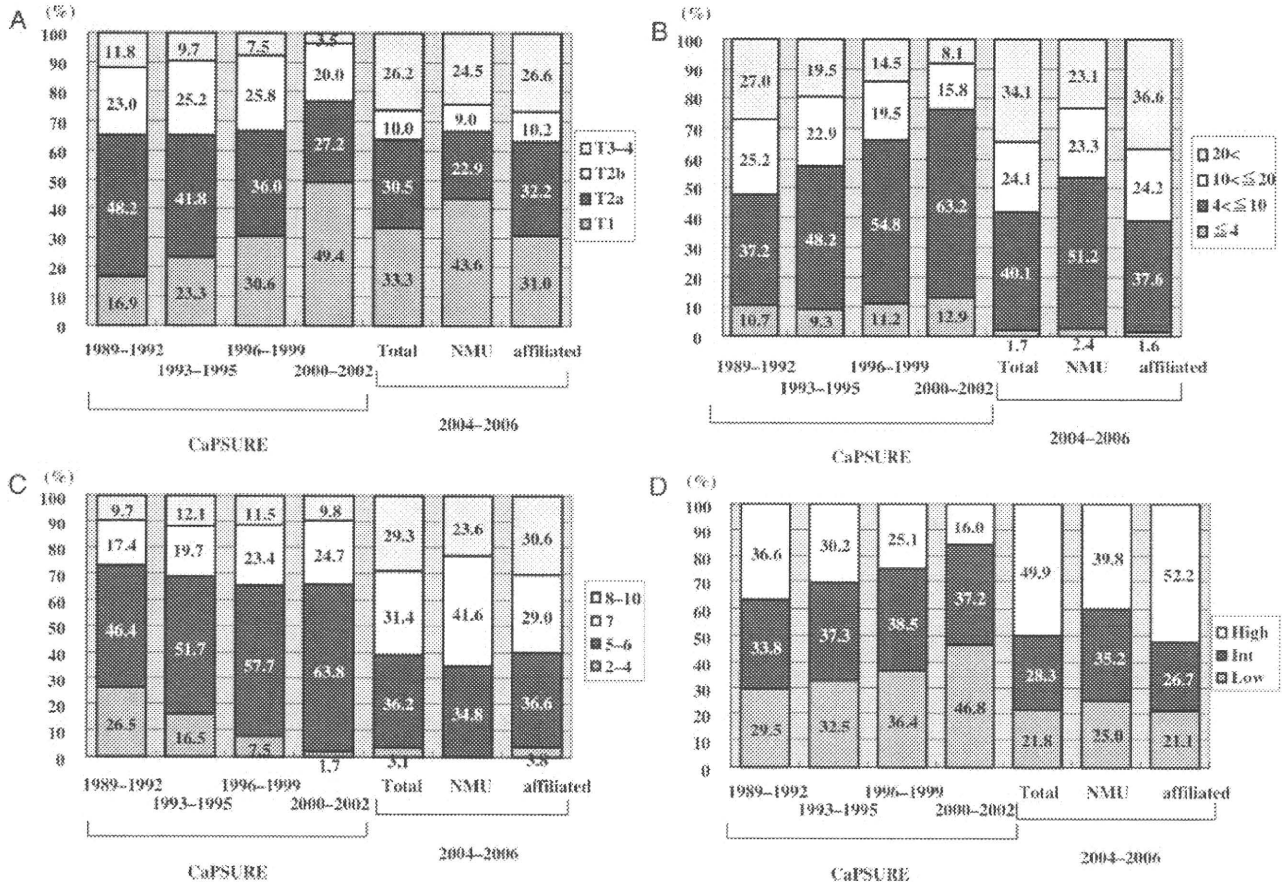

Figure 1. The chronological variation in the clinical T stage (A), PSA value (B), Gleason score (C) and risk classification (D) of the CaPSURE data (1) in comparison with those of our present study. (CaPSURE data cited by Cooperberg et al. (1).) NMU, Nara Medical University; affiliated, affiliated hospitals.

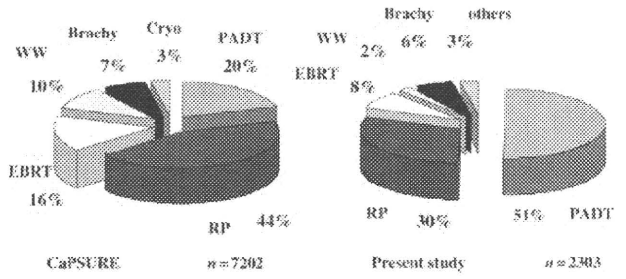

Figure 2. The distribution of primary therapy in our present study and the CaPSURE data (4). (CaPSURE data cited by Cooperberg et al. (1).) PADT, primary androgen deprivation therapy; RP, radical prostatectomy; EBRT, external beam radiation therapy; WW, watchful waiting; Brachy, seed implant; Cryo, cryosurgery. ($P < 0.001$, chi-square test).

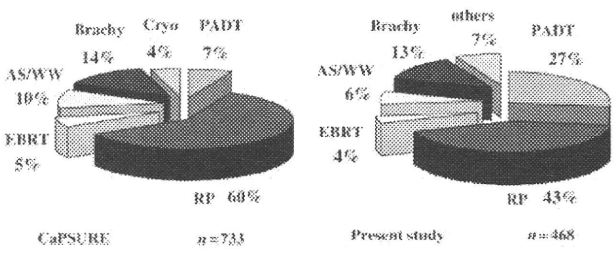

Figure 3. The distribution of primary therapy in patients with low risk in our present study and the CaPSURE data (7) (CaPSURE data cited by Cooperberg et al. (7).) PADT, primary androgen deprivation therapy; RP, radical prostatectomy; EBRT, external beam radiation therapy; AS/WW, active surveillance or watchful waiting; Brachy, seed implant; Cryo, cryosurgery. ($P < 0.001$, chi-square test).

diagnosed. The physicians in the affiliated hospitals are also likely to perform primary therapy for their patients. Indeed, the proportion of PADT was higher in the institutes where EBRT was unavailable than in those where EBRT was available as in our institute (data not shown). Furthermore, the number of RP per year in each institute correlated inversely with the proportion of PADT (data not shown).

In the last decade, most urologists conducted RP for patients with localized or locally advanced prostate cancer. In Japan, permanent seed implantation was legally approved in 2003. NMU has started seed implantation since 2004 (11), and IMRT was also started since 2005. The number of patients receiving seed implant and IMRT has increased year by year. Most physicians have recognized the usefulness of RT for localized prostate cancer. In the near future, the trend of primary therapy for prostate cancer will be expected to change. Nonetheless, we emphasize that we still treat a great proportion of the high-risk patients in Japan.

CONCLUSION

The present study clarified the differences in the characteristics of prostate cancer patients between the USA and Japan. The Japanese patients with prostate cancer still have higher PSA at diagnosis, higher Gleason score and higher clinical stage than the American patients. In other words, the Japanese patients to whom urologists face up were not similar to patients with prostate cancer in the USA. Therefore, the trend of primary therapy for prostate cancer in Japan was different from that in the USA. The higher rate of PADT is the most characteristic for the Japanese patients.

Conflict of interest statement

None declared.

References

1. Cooperberg MR, Lubeck DP, Mehta SS, Carroll PR. Time trends in clinical risk stratification for prostate cancer: implications for outcomes (data from CaPSURE). *J Urol* 2003;170:S21–S27.
2. Sirovich BE, Schwartz LM, Woloshin S. Screening men for prostate and colorectal cancer in the United States: does practice reflect the evidence? *J Am Med Assoc* 2003;289:1414–20.
3. Ito K, Kakehi Y, Naito S, Okuyama A, Japanese Urological Association. Japanese Urological Association guidelines on prostate-specific antigen-based screening for prostate cancer and the ongoing cluster cohort study in Japan. *Int J Urol* 2008;15:763–8.
4. Cooperberg MR, Lubeck DP, Penson DF, Mehta SS, Carroll PR, Kane CJ. Sociodemographic and clinical risk characteristics of patients with prostate cancer within the Veterans Affairs health care system: data from CaPSURE. *J Urol* 2003;170:905–8.
5. Cancer Registration Committee of the Japanese Urological Association. Clinicopathological statistics on registered prostate cancer patients in Japan: 2000 report from the Japanese Urological Association. *Int J Urol* 2005;12:46–61.
6. D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *J Am Med Assoc* 1998;280:969–74.
7. Cooperberg MR, Broering JM, Kantoff PW, Carroll PR. Contemporary trends in low risk prostate cancer: risk assessment and treatment. *J Urol* 2007;178:S14–S19.
8. Akaza H, Usami M, Hinotsu S, Ogawa O, Kagawa S, Kitamura T, et al. Characteristics of patients with prostate cancer who have initially been treated by hormone therapy in Japan: J-CaP surveillance. *Jpn J Clin Oncol* 2004;34:329–36.
9. Akaza H. Trends in primary androgen depletion therapy for patients with localized and locally advanced prostate cancer: Japanese perspective. *Cancer Sci* 2006;97:243–7.
10. Akaza H, Hinotsu S, Usami M, Ogawa O, Kagawa S, Kitamura T, et al. The case for androgen deprivation as primary therapy for early stage disease: results from J-CaP and CaPSURE. *J Urol* 2006;176:S47–S49.
11. Tanaka N, Asakawa I, Kondo H, Tanaka M, Fujimoto K, Hasegawa M, et al. Technical acquisition and dosimetric assessment of iodine-125 permanent brachytherapy in localized prostate cancer: our first series of 100 patients. *Int J Urol* 2009;16:70–4.

Clinical Significance of Polymorphism and Expression of Chromogranin A and Endothelin-1 in Prostate Cancer

Zhiyong Ma, Norihiko Tsuchiya,* Takeshi Yuasa, Mingguo Huang, Takashi Obara, Shintaro Narita, Yohei Horikawa, Hiroshi Tsuruta, Mitsuru Saito, Shigeru Satoh, Osamu Ogawa and Tomonori Habuchi

From the Department of Urology, Akita University School of Medicine (ZM, NT, MH, TO, SN, YH, HT, MS, SS, TH), Akita, Department of Medical Oncology and Genitourinary Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research (TY), Tokyo and Department of Urology, Graduate School of Medicine, Kyoto University (OO), Kyoto, Japan

Abbreviations and Acronyms

BPH = benign prostatic hyperplasia
BPHcont = nonprostate cancer specimen BPH region
BPHpca = prostate cancer specimen BPH region
CHGA = chromogranin A
ET = endothelin
IHC = immunohistochemistry
LD = linkage disequilibrium
PCapca = prostate cancer region
PCR = polymerase chain reaction
PSA = prostate specific antigen
RRP = radical retropubic prostatectomy

Submitted for publication October 17, 2009.
Study received institutional ethics committee approval.
Supported by Japan Society for Promotion of Science, Japan (18390411, 19591832, 19591833, 19659406 and 21592029).
* Correspondence: Department of Urology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan (FAX: 81-18-836-2619; e-mail: tsuchiya@med.akita-u.ac.jp).

Purpose: We investigated the clinical significance of chromogranin A and endothelin-1 polymorphism and expression in prostate cancer.
Materials and Methods: We analyzed 2 *CHGA* polymorphisms by polymerase chain reaction-restriction fragment length polymorphism in DNA samples of 435 patients with prostate cancer and 316 age matched male controls. Chromogranin A and endothelin-1 expression was evaluated by immunohistochemistry in prostate specimens of 114 men with prostate cancer who underwent radical retropubic prostatectomy and in 27 with bladder cancer who underwent radical cystectomy and served as controls.
Results: For the *CHGA* Glu264Asp polymorphism men with the *GG* genotype were at 2.05 times higher risk for prostate cancer than men with the *CC* genotype ($p = 0.014$). In men with prostate cancer higher chromogranin A immunohistochemistry grade was associated with higher stage and higher Gleason score ($p = 0.011$ and 0.044, respectively). Multivariate analysis showed that chromogranin A immunohistochemistry grade was an independent variable for predicting biochemical failure after radical prostatectomy ($p = 0.023$). Higher endothelin-1 expression was observed in prostate cancers ($p = 0.011$), especially those with a higher Gleason score ($p = 0.042$). There was no significant relationship between chromogranin A polymorphisms, and chromogranin A and endothelin-1 expression.
Conclusions: Polymorphism and expression of chromogranin A and endothelin-1 have clinical significance in prostate cancer. Chromogranin A expression was an independent predictor of biochemical failure after prostatectomy in patients with localized prostate cancer.

Key Words: prostate; prostatic neoplasms; polymorphism, genetic; chromogranin A; endothelin-1

NEUROENDOCRINE cells have an important role in normal prostates and BPH as well as in primary and metastatic prostate cancer.[1,2] Of the biogenic amines and neuropeptides secreted by neuroendocrine cells CHGA is a candidate marker for diagnosing and predicting the prognosis of prostate cancer. Patients with prostate cancer have significantly higher serum CHGA than those with BPH and controls.[2] A group reported that CHGA protein expression determined by IHC is a useful prognostic marker of biochemical failure after radical prostatectomy.[3] To date only 1 group

0022-5347/10/1843-1182/0
THE JOURNAL OF UROLOGY®

Vol. 184, 1182-1188, September 2010
Printed in U.S.A.
DOI:10.1016/j.juro.2010.04.063

has performed IHC analysis and found higher CHGA expression in benign epithelial cells adjacent to prostate cancer lesions than in the BPH region.[4] On the other hand, CHGA polymorphisms can influence CHGA expression, which eventually affects baseline blood pressure,[5] but the relationship between CHGA polymorphisms and prostate cancer remains unclear.

ETs, which are endogenous small peptides secreted by endothelium, exert paracrine and autocrine effects through cell surface receptors and influence cellular processes, such as angiogenesis, cellular proliferation, and tissue repair and development.[6–8] Plasma ET-1 levels in patients with hormone refractory, metastatic prostate cancer are higher than in patients with organ confined prostate cancer or controls.[9] Another IHC study showed ET-1 over expression in cases of advanced prostate cancer and high grade prostatic intraepithelial neoplasia.[10] Recently CHGA and ET-1 interaction was reported in a group of twins as well as in vitro experiments.[11] The study showed that polymorphisms in the CHGA promoter region are associated with serum ET-1 and CHGA stimulated ET-1 secretion in endothelial cells in a dose dependent manner. To our knowledge the association between CHGA and ET-1 in prostate cancer has not been assessed.

We analyzed 7 polymorphisms in the promoter region and the Glu264Asp polymorphism in exon 6 of *CHGA* in a Japanese population to evaluate the relationship to prostate cancer risk and clinical characteristics. We evaluated CHGA and ET-1 protein expression to determine whether they are related to localized prostate cancer pathological features and treatment outcomes. Also, we assessed the relationships among the *CHGA* genotypes, CHGA protein expression and ET-1 protein expression.

MATERIALS AND METHODS

Subjects

A total of 751 men, including 435 with prostate cancer and 316 controls, were enrolled in this study. All patients with prostate cancer were diagnosed at Akita University Medical Center, Kyoto University affiliated hospital and related community hospitals. They were pathologically diagnosed using specimens obtained from transrectal needle biopsy or transurethral prostate resection due to lower urinary tract symptoms. Prostate cancer clinical or pathological stage at diagnosis was determined by reviewing the medical records based on the TNM system. Prostate cancer was classified as stage A—T1a-bN0M0, stage B—T1c-2N0M0, stage C—T3-4N0M0 and stage D—T1-4N1M0-1 or T1-4N0-1M1 by the modified Whitmore-Jewett system. Controls were native Japanese men older than 60 years who had undergone health inspection at a community hospital.

IHC was done in prostate specimens from 114 men with stage T2-4 prostate cancer who underwent RRP and in BPH specimens from 27 who underwent radical cystectomy for bladder cancer. Since endocrine therapy may affect the number of neuroendocrine cells, patients with prostate cancer treated with endocrine therapy before RRP were excluded from analysis.[12] Clinical information was reviewed in the medical records. DNA and prostate specimens were collected after obtaining informed consent with approval from the institutional ethics committee.

CHGA Polymorphism Genotyping

We selected 7 polymorphisms in the *CHGA* promoter region for LD analysis. DNA direct sequencing was done in 200 samples to analyze the genotypes of those polymorphisms. The Appendix lists PCR primer sequences. Genotype data were imported into Haploview, version 3.32 (Daly Laboratory, Board Institute, Cambridge, Massachusetts) to test LD among polymorphisms in the *CHGA* promoter region. D′ greater than 0.8 was considered a strong LD.

Finally, we analyzed 2 *CHGA* polymorphisms, including rs9658635 in the promoter region and Glu264Asp in exon 6, using certain primers (table 1). After confirming successful PCR amplification each product was digested at 37C overnight with 5 U *Bcc* I or *BfuC* I restriction enzymes (New England Biolabs, Beverly, Massachusetts). For the rs9658635 polymorphism restriction fragments were 114 and 21 bp for the *T* allele, and 135 bp for the *C* allele. For the Glu264Asp polymorphism restriction fragments were 129 and 106 bp for the *G* allele, and 235 bp for the *C* allele. To avoid genotyping errors caused by incomplete digestion or other technical failures we repeated the experiment at least twice for all samples and compared the genotype with the DNA sequencing results in 100 randomly selected samples.

Table 1. *PCR primers*

	Reference Single Nucleotide Polymorphism	
	rs9658635	rs9658655
Polymorphism	T-415C	Glu264Asp
Primers	Forward-5′ CCTAGATATTGGAGAGAGCCATGAGTGA 3′ Reverse-5′ CCATGTGTACTGAGGTCCCTGGCAG 3′	Forward-5′ AGGGTGGCAGGCAAAGAG 3′ Reverse-5′ AAGGTGGAATGAGGTTATGG 3′
Length (bp)	135	235
Enzyme	*BccI*	*BfuCI*
Fragments (bp)	21 + 114	106 + 129

IHC Staining and Evaluation

We performed IHC staining for CHGA and ET-1 using a certain protocol. Briefly, deparaffinized, rehydrated sections were steamed for 20 minutes to enhance antigen retrieval. Immunohistochemical labeling with mouse anti-human CHGA antibody (DakoCytomation, Glostrup, Denmark) (×800) or ET-1 antibody (Alexis Biochemicals, Lausen, Switzerland) (×250) was done overnight at 4C. Slides were labeled with the anti-mouse EnVision™+ system labeled with horseradish peroxidase for 30 minutes. The liquid DAB+ Substrate-Chromogen System (DakoCytomation) was applied at room temperature for 30 minutes. Slides were counterstained with hematoxylin solution for nuclear staining. Specimens were examined by 2 independent researchers blinded to sample background data.

CHGA positive stained cells were counted in 10 high power visual fields at 200× magnification to determine which had the most positive cells (fig. 1, *A*). Since the number of CHGA positive cells in the BPH region was greatly different than that in the prostate cancer region, CHGA positive cells in 3 regions were counted, including BPHcont, BPHpca and PCapca. Counting was done 3 times per sample and the mean was used for statistics. The mean value of each sample was categorized as grade 1—less than 10, grade 2—10 to 29 and grade 3—30 or greater for the prostate cancer region. Since neuroendocrine cells are consistently found in the periurethral ducts and verumontanum,[13] those regions were excluded from counting.

Cytoplasmic ET-1 staining intensity was scored on a semiquantitative scale as 1—weak, 2—moderate and 3—strong (fig. 1, *B*). The percent of cytoplasmic ET-1 positive cells was divided into 4 groups, including 1—less than 25%, 2—25% to 50%, 3—50% to 75% and 4—greater than 75%. Total immunoreactivity grade was calculated by multiplying the 2 scores[14] and defined as grade 1—6 or less, grade 2—8 and grade 3—greater than 8.

Statistical Analysis

All data were entered into an Access® database and analyzed using Excel® 2007 and SPSS®, version 16.0J. We examined differences in mean age in the 3 groups using the independent t test. Hardy-Weinberg equilibrium analysis was done to compare observed and expected genotype frequency using the Pearson chi-square test. We used binary logistic regression to assess the association between prostate cancer risk and genotypes by calculating the OR and 95% CI. We hypothesized that the *C* allele of the rs9658635 polymorphism would be an inherent genetic risk factor for prostate cancer and prostate cancer progression. Statistical modeling was done independently on the relative risk of the *CC* or *CT* genotype against the *TT* genotype for rs9658635 using the logistic regression model adjusted by age. For Glu264Asp the *G* allele was hypothesized as an inherent genetic risk factor for prostate cancer and prostate cancer progression.

We used 1-way ANOVA to compare the number of CHGA IHC positive cells among the 3 groups and Kendall's τ-b rank correlation coefficients to examine the relationship between IHC grade and Gleason score or clinical stage. The biochemical failure-free interval was defined as the time from the date of RRP to the date when PSA increased to more than 0.4 ng/ml. We estimated relationships between polymorphisms or IHC grade and biochemical failure-free survival in stage T2-4 prostate cancer cases by the Kaplan-Meier method and evaluated them by the log rank test. The Cox multivariate proportional hazards model was used for multivariate analysis. We examined relationships between polymorphisms and IHC grades in patients with prostate cancer using Fisher's exact test. All statistical tests and p values were 2-tailed with results considered significant at $p < 0.05$.

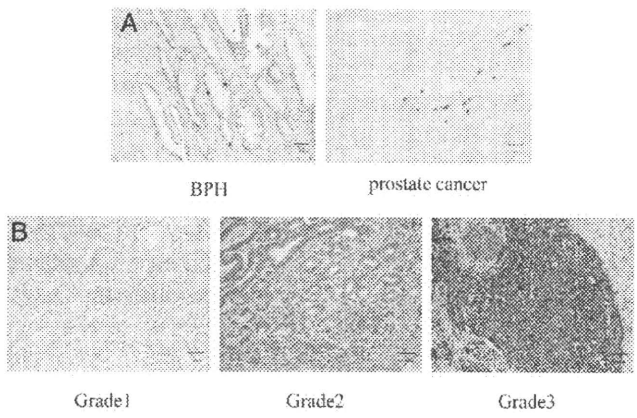

Figure 1. IHC in BPH and prostate cancer regions. *A*, CHGA cytoplasmic staining pattern. *B*, representative ET-1 IHC stains of different grades.

RESULTS

Characteristics

Mean age ± SD in patients with prostate cancer and male controls was 70.28 ± 7.43 and 69.46 ± 7.22 years, respectively ($p = 0.289$). Stage was A to C, D1 and D2 in 10, 191, 83, 25 and 126 patients with prostate cancer, respectively. In the prostate cancer group Gleason score was less than 7, 7, greater than 7 and unavailable in 14, 202, 164 and 55 patients, respectively.

CHGA Associations

Polymorphism genotypes vs prostate cancer risk and clinicopathological factors. Genotype distributions in all groups were consistent with Hardy-Weinberg equilibrium. Since more than 90% of D' values in the 7 polymorphisms in the *CHGA* promoter region equaled 1 (fig. 2), the rs9658635 polymorphism, which was reported to be associated with CHGA expression,[5] was chosen as a representative polymorphism for further analysis. Statistical analysis of genotype frequency showed no relationship between the rs9658635 polymorphism and the prostate cancer risk ($p > 0.05$, table 2). For the Glu264Asp polymorphism we found a significantly increased prostate cancer risk in men with the *GG*

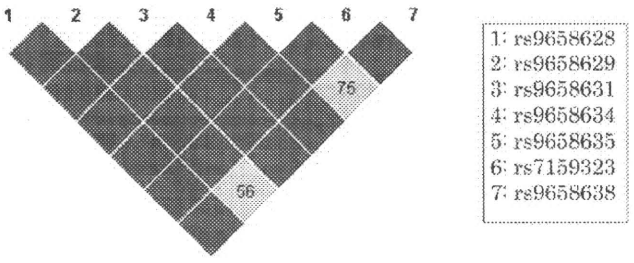

Figure 2. There were strong LDs among 7 CHGA promoter region polymorphisms. Red diamonds indicate D' = 1. Gray diamonds indicate D' less than 1.

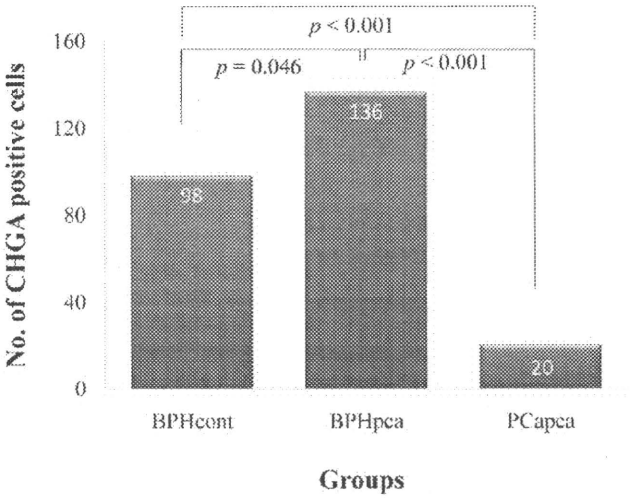

Figure 3. There were significant differences among mean number of CHGA IHC positive cells in BPHcont, BPHpca and PCapca.

genotype and the *GC* genotype than in those with the *CC* genotype (OR 2.05, 95% CI 1.16–3.63; p = 0.014 and OR 1.97, 95% CI 1.10–3.52; p = 0.023, respectively, table 2). There was no significant association of the rs9658635 or the Glu264Asp *CHGA* polymorphism with prostate cancer clinical stage or Gleason score (p >0.05).

IHC grade vs prostate cancer clinicopathological factors and prognosis. BPHcont, BPHpca and PCapca showed a mean ± SD of 97 ± 81, 136 ± 109 and 20 ± 48 CHGA IHC positive cells, respectively (p <0.001, fig. 3). Compared with BPHcont BPHpca had more and PCapca had fewer CHGA positive cells (p = 0.046 and <0.001, respectively). In patients with prostate cancer a higher CHGA IHC grade was more often found in those with pT3-4 than pT2 disease (p = 0.011). There was a significant association between CHGA IHC grade and Gleason score (p = 0.044). On univariate analysis a higher probability of biochemical failure after RRP was significantly associated with higher CHGA IHC grade (p = 0.001, fig. 4), higher Gleason score (p = 0.039), higher stage (p = 0.025) and higher PSA at diagnosis (p <0.001). On multivariate analysis CHGA IHC grade was an independent factor predicting possible biochemical failure after RRP (p = 0.023, table 3).

ET-1 IHC Grade

Prostate cancer showed a higher ET-1 IHC grade than BPH (chi-square 9.030, p = 0.011). Of patients with prostate cancer we noted a higher ET-1 IHC grade in those with a higher Gleason score (chi-square 4.149, p = 0.042, fig. 5). There was no statistically significant relationship between ET-1 IHC grade and clinical stage or biochemical failure after RRP (p = 0.661 and 0.230, respectively).

CHGA Polymorphism Genotypes vs CHGA and ET-1

Cross-tabulation results showed no significant association of the *CHGA* rs9658635 or the Glu264Asp polymorphism with CHGA or ET-1 IHC grade (table 4). We found no significant relationship between CHGA and ET-1 expression (p >0.05).

Table 2. *CHGA polymorphisms vs prostate cancer risk and clinicopathological factors*

	Prostate Ca vs Control		Clinical Stage D vs A + B + C		Gleason Score 8 or Greater vs Less Than 8	
Genotype Polymorphism	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
CHGA promoter rs9658635:						
TT	1		1		1	
CT	1.12 (0.81–1.55)	0.502	1.41 (0.87–2.31)	0.167	1.26 (0.79–1.98)	0.331
CC	0.83 (0.55–1.25)	0.363	1.24 (0.65–2.39)	0.512	1.03 (0.56–1.91)	0.914
CT + CC	1.02 (0.76–1.38)	0.892	1.37 (0.86–2.18)	0.187	1.19 (0.77–1.84)	0.424
TT + CT:CC	1.29 (0.89–1.86)	0.185	0.98 (0.55–1.76)	0.954	1.10 (0.64–1.91)	0.729
Exon 6 Glu264Asp:						
CC	1		1		1	
GC	1.97 (1.10–3.52)	0.023	1.80 (0.64–5.10)	0.268	2.98 (0.96–9.22)	0.059
GG	2.05 (1.16–3.63)	0.014	2.11 (0.76–5.87)	0.154	2.62 (0.86–8.04)	0.091
GC + CC	2.01 (1.15–3.51)	0.014	1.98 (0.72–5.43)	0.817	2.77 (0.91–8.37)	0.072
CC + GC:GG	0.86 (0.64–1.15)	0.314	0.80 (0.54–1.20)	0.279	1.02 (0.69–1.51)	0.904

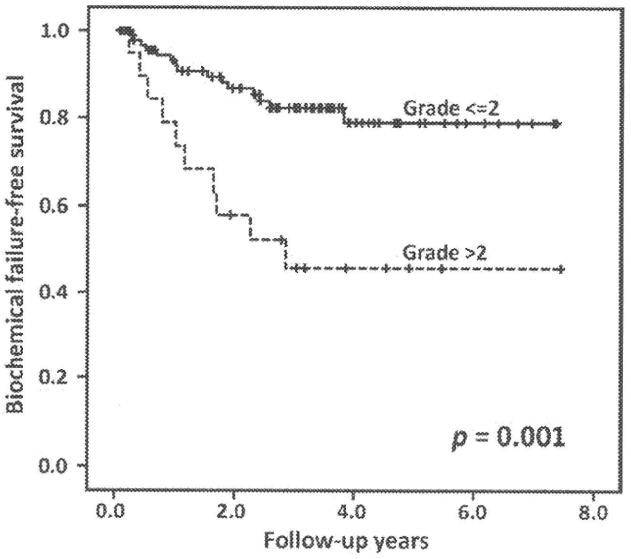

Figure 4. Higher CHGA IHC grade was associated with higher probability of biochemical failure after RRP.

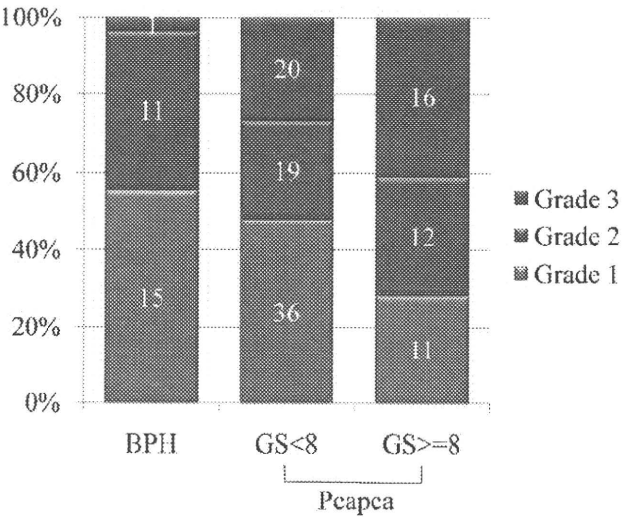

Figure 5. Compared with BPH prostate cancer showed higher ET-1 IHC grade. Higher ET-1 IHC grade was associated with higher Gleason score (*GS*) prostate cancer.

DISCUSSION

We first investigated the influence of CHGA polymorphisms on prostate cancer clinicopathological factors. Results revealed a significant association between the *G* allele of the *CHGA* Glu264Asp polymorphism and the risk of prostate cancer in a native Japanese population, suggesting that the *CHGA* Glu264Asp polymorphism may be a useful marker for estimating the prostate cancer risk. To our knowledge this is the first study to investigate whether *CHGA* gene variants influence prostate cancer. The *G* to *C* allele variant of the Glu264Asp polymorphism caused the 264 amino acid CHGA to change from glutamic to aspartic acid. Pancreastatin, an impairing glucose metabolism peptide of 52 amino acids, is located in this CHGA encoding region.[15] Pancreastatin inhibits the release of glucose stimulated insulin from pancreatic islet β cells.[16] Since insulin has an important role in prostate cancer pathogenesis,[17] it is reasonable that the *CHGA* Glu264Asp polymorphism affects prostate cancer carcinogenesis through functional alteration of pancreastatin by regulating insulin secretion. Also, the importance of the pancreastatin polymorphism was supported by the observation that the Gly297Ser polymorphism influences glucose uptake.[18] Pancreastatin is a useful prognostic indicator in patients with neuroendocrine tumors[19] but there was no association between the CHGA Glu264Asp polymorphism and prostate cancer prognosis.

The CHGA rs9658634 polymorphism is reportedly associated with serum CHGA and the ET-1 level.[5,11] However, the CHGA polymorphism showed no relationship with CHGA or ET-1 IHC expression in our study, which could have been due to several reasons. 1) The different methods of measuring CHGA expression may have led to different results. Since CHGA is secreted by other tissues as well as the prostate, serum CHGA represents total CHGA expression in the whole body. We used prostate surgical specimen IHC grade instead of the serum level to more specifically reflect prostate CHGA expression in the prostate. In support of our findings another research group found no correlation between serum CHGA and immunohistochemical results.[20] 2) The discrepancy may have been be due to our population. Only men older than 60 years with prostate cancer were enrolled in our study

Table 3. *Biochemical failure and clinical factor Cox proportional hazards model*

Clinical Factors	Univariate		Multivariate	
	HR (95% CI)	p Value	HR (95% CI)	p Value
CHGA IHC grade greater than 2 or not	3.585 (1.609–7.987)	0.002	2.713 (1.149–6.407)	0.023
Gleason score 8 or greater or not	2.237 (1.020–4.908)	0.044	1.512 (0.650–3.517)	0.337
T stage greater than 2 or not	2.422 (1.088–5.394)	0.030	0.969 (0.375–2.504)	0.948
PSA greater than 10 ng/ml or not	5.682 (1.950–16.557)	0.001	4.611 (1.483–14.336)	0.008

Table 4. *CHGA polymorphisms vs CHGA and ET-1 expression*

	CHGA IHC Grade				ET-1 IHC Grade			
	1	2	3	p Value	1	2	3	p Value
rs9658635:				0.964				0.497
TT	29	6	9		19	14	11	
CT	32	10	9		18	14	19	
CC	13	3	3		10	3	6	
Glu264Asp:				0.423				0.272
CC	4	0	0		1	2	1	
GC	27	11	10		17	17	14	
GG	43	8	11		29	12	21	

whereas other studies included subjects without cancer regardless of age or gender. This may result in the lack of a significant relationship between CHGA polymorphisms and expression in patients with prostate cancer.

However, our study shows that in patients with localized prostate cancer and no history of endocrine therapy a higher CHGA IHC grade was associated with worse tumor stage and higher Gleason score. A group also reported that IHC staining for CHGA is significantly associated with Gleason score,[20] although a contradictory result was reported.[21] Furthermore, the Cox multivariate regression model showed that CHGA IHC grade was an independent variable for predicting biochemical failure after RRP. In a study of lymph node positive cases of prostate cancer the investigators found that CHGA expression is associated with biochemical failure after RRP.[22] However, the history of endocrine therapy before RRP, which was associated with CHGA expression,[12] was not controlled in that study. Other studies in D2 prostate cancer cases showed that higher CHGA IHC grade is associated with a worse prognosis.[21,23] Taken together, CHGA IHC grade, which represents neuroendocrine differentiation, could predict the prognosis in patients with prostate cancer.

Compared with BPHcont, the number of CHGA IHC positive cells was higher in BPHpca and lower in PCapca (fig. 3). This agrees with the result that CHGA positive cells had more prominent expression in benign epithelial cells adjacent to prostate cancer lesions than in the prostate cancer region.[4] Also, there is a tendency toward a decreased number of neuroendocrine cells in untreated patients with prostate cancer compared with that in patients with BPH and male controls with a normal prostate.[24] Hence, higher serum CHGA in patients with prostate cancer may result from BPHpca, which has many more neuroendocrine cells than in patients with BPH. This indicates that to predict prostate cancer susceptibility more efficiently we should focus on the cancerous region and the adjacent noncancerous region.

Neuroendocrine cells in the BPH region are negative for α-methylacyl coenzyme A racemase while neuroendocrine cells in prostate cancer are positive for α-methylacyl coenzyme A racemase.[25] In vitro cells of the androgen dependent line LNCaP were induced to show neuroendocrine differentiation by androgen deprivation[26] or agents that increase intracellular cyclic adenosine monophosphate.[27] Results indicate that PCapca neuroendocrine cells, which have hormone insensitive characteristics, may differentiate from prostate cancer cells. Patients with the worst prostate cancer stages had a higher CHGA IHC grade, indicating that more prostate cancer cells had transformed into neuroendocrine cells with hormone insensitive characteristics and resulting in a worse prognosis. Whether our patients with hormone refractory prostate cancer had many more hormone insensitive neuroendocrine cells than our patients with localized prostate cancer should be explored in the future.

Serum ET-1 has no value for estimating prostate cancer prognosis.[28] We noted no association of ET-1 expression with clinical stage or biochemical failure after RRP. However, prostate cancer showed significantly higher ET-1 expression than the BPH region and higher ET-1 IHC grade was associated with a higher Gleason score.

CONCLUSIONS

A *CHGA* genetic variant may modify prostate cancer carcinogenesis and CHGA expression may be a useful biomarker to predict the higher malignant potential of localized prostate cancer and biochemical failure after RRP. Thus, results suggest that CHGA is involved in prostate cancer carcinogenesis and progression.

ACKNOWLEDGMENTS

Mrs. Mitobe provided technical assistance.

APPENDIX

DNA Sequencing Primers for CHGA Promoter Region

Fragment No. (polymorphisms)	Reference Single Nucleotide Polymorphism	Primers	
		PCR	DNA Sequencing
1:		Forward-5′ CAGGTTCTCATTTAGGGACA 3′ Reverse-5′ AAAGGTCAGTTTCCTGGTTG 3′	Forward-5′ TTTAGGGACAGGCGTGAGCACAGGT 3′ Reverse-5′ TCAGTTTCCTGGTTGGCTTCCCTT 3′
G-1106A	rs9658628		
A-1018T	rs9658629		
T-998G	rs9658631		
2:		Forward-5′ CATCAGTTACCTGTCAAGTGCGT 3′ Reverse-5′ CCCCGTGCTATTTTTCCTAAGT 3′	Forward-5′ TGTCAAGTGCGTTTCCTCTGT 3′ Reverse-5′ TTCCTAAGTGCCCTCTGCCT 3′
G-462A	rs9658634		
T-415C	rs9658635		
3:		Forward-5′ GCCCAGGGACACAAGGCAAAT 3′ Reverse-5′ TCGGCGTGCGTCCGTCTGTC 3′	Forward-5′ CACCTCTTGGAAACCAGATACC 3′ Reverse-5′ TGCGTCCGTCTGTCGGTCGATG 3′
C-89A	rs7159323		
C-57T	rs9658638		

REFERENCES

1. di Sant'Agnese PA and de Mesy Jensen KL: Neuroendocrine differentiation in prostatic carcinoma. Hum Pathol 1987; **18:** 849.
2. Ranno S, Motta M, Rampello E et al: The chromogranin-A (CgA) in prostate cancer. Arch Gerontol Geriatr 2006; **43:** 117.
3. May M, Siegsmund M, Hammermann F et al: Prognostic significance of proliferation activity and neuroendocrine differentiation to predict treatment failure after radical prostatectomy. Scand J Urol Nephrol 2007; **41:** 375.
4. Sion-Vardy N, Tzikinovsky A, Bolotyn A et al: Augmented expression of chromogranin A and serotonin in peri-malignant benign prostate epithelium as compared to adenocarcinoma. Pathol Res Pract 2004; **200:** 493.
5. Chen Y, Rao F, Rodriguez-Flores JL et al: Common genetic variants in the chromogranin A promoter alter autonomic activity and blood pressure. Kidney Int 2008; **74:** 115.
6. Schiffrin EL, Deng LY, Sventek P et al: Enhanced expression of endothelin-1 gene in resistance arteries in severe human essential hypertension. J Hypertens 1997; **15:** 57.
7. Li JS, Lariviere R and Schiffrin EL: Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats. Evidence for a role of endothelin in vascular hypertrophy. Hypertension 1994; **24:** 183.
8. Kopetz ES, Nelson JB and Carducci MA: Endothelin-1 as a target for therapeutic intervention in prostate cancer. Invest New Drugs 2002; **20:** 173.
9. Nelson JB, Hedican SP, George DJ et al: Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat Med 1995; **1:** 944.
10. Montironi R, Mazzucchelli R, Barbisan F et al: Immunohistochemical expression of endothelin-1 and endothelin-A and endothelin-B receptors in high-grade prostatic intraepithelial neoplasia and prostate cancer. Eur Urol 2007; **52:** 1682.
11. Lillie EO, Mahata M, Khandrika S et al: Heredity of endothelin secretion: human twin studies reveal the influence of polymorphism at the chromogranin A locus, a novel determinant of endothelial function. Circulation 2007; **115:** 2282.
12. Ahlgren G, Pedersen K, Lundberg S et al: Regressive changes and neuroendocrine differentiation in prostate cancer after neoadjuvant hormonal treatment. Prostate 2000; **42:** 274.
13. di Sant'Agnese PA, de Mesy Jensen KL, Churukian CJ et al: Human prostatic endocrine-paracrine (APUD) cells. Distributional analysis with a comparison of serotonin and neuron-specific enolase immunoreactivity and silver stains. Arch Pathol Lab Med 1985; **109:** 607.
14. Liao Y, Abel U, Grobholz R et al: Up-regulation of insulin-like growth factor axis components in human primary prostate cancer correlates with tumor grade. Hum Pathol 2005; **36:** 1186.
15. Taupenot L, Harper KL and O'Connor DT: The chromogranin-secretogranin family. N Engl J Med 2003; **348:** 1134.
16. Sanchez-Margalet V, Gonzalez-Yanes C, Santos-Alvarez J et al: Pancreastatin. Biological effects and mechanisms of action. Adv Exp Med Biol 2000; **482:** 247.
17. Nandeesha H: Insulin: a novel agent in the pathogenesis of prostate cancer. Int Urol Nephrol 2008; **41:** 267.
18. O'Connor DT, Cadman PE, Smiley C et al: Pancreastatin: multiple actions on human intermediary metabolism in vivo, variation in disease, and naturally occurring functional genetic polymorphism. J Clin Endocrinol Metab 2005; **90:** 5414.
19. Stronge RL, Turner GB, Johnston BT et al: A rapid rise in circulating pancreastatin in response to somatostatin analogue therapy is associated with poor survival in patients with neuroendocrine tumours. Ann Clin Biochem 2008; **45:** 560.
20. Grimaldi F, Valotto C, Barbina G et al: The possible role of chromogranin A as a prognostic factor in organ-confined prostate cancer. Int J Biol Markers 2006; **21:** 229.
21. Kokubo H, Yamada Y, Nishio Y et al: Immunohistochemical study of chromogranin A in stage D2 prostate cancer. Urology 2005; **66:** 135.
22. Quek ML, Daneshmand S, Rodrigo S et al: Prognostic significance of neuroendocrine expression in lymph node-positive prostate cancer. Urology 2006; **67:** 1247.
23. Kamiya N, Suzuki H, Kawamura K et al: Neuroendocrine differentiation in stage D2 prostate cancers. Int J Urol 2008; **15:** 423.
24. Guate JL, Escaf S, Menendez CL et al: Neuroendocrine cells in benign prostatic hyperplasia and prostatic carcinoma: effect of hormonal treatment. Urol Int 1997; **59:** 149.
25. Huang J, Yao JL, di Sant'Agnese PA et al: Immunohistochemical characterization of neuroendocrine cells in prostate cancer. Prostate 2006; **66:** 1399.
26. Burchardt T, Burchardt M, Chen MW et al: Transdifferentiation of prostate cancer cells to a neuroendocrine cell phenotype in vitro and in vivo. J Urol 1999; **162:** 1800.
27. Cox ME, Deeble PD, Lakhani S et al: Acquisition of neuroendocrine characteristics by prostate tumor cells is reversible: implications for prostate cancer progression. Cancer Res 1999; **59:** 3821.
28. Zhou WQ, Sun YH, Xu CL et al: Plasma endothelin-1 in patients with prostate cancer and its clinical significance. Zhonghua Nan Ke Xue 2006; **12:** 315.

原 著

小切開による拡大前立腺全摘術

川島 清隆

栃木県立がんセンター泌尿器科*

Extended radical prostatectomy by minimum incision

Kiyotaka Kawashima
Department of Urology, Tochigi Cancer Center

Abstract

Objectives : We have performed extended radical prostatectomy for high-risk prostate cancer by minimal incision in all cases since 2007. The operative technique and treatment results are reported. Material and methods : Extended radical prostatectomy was performed in 198 cases during the period from April 2007 through July 2009. The most important point of this procedure is removing the prostate without exposing prostate surface at all surface. En-block removal of the prostate is required. We perform "Initial exposure of the urethra" technique for apical dissection and "Complete exposure of the longitudinal muscle of the rectum" for dorsal dissection. Result : Over all positive surgical margin rate was 12.9%. Positive surgical margin rate in pT2 was only 1.8%. Positive surgical margin rate in pT3 was 27.1%. Average blood loss was 354ml (63 ～ 2,204ml). Conclusion : Extended radical prostatectomy by minimal incision was thought to be good for high cancer control not only for good QOL.

Key words : minimum incision endoscopic surgery, radical prostatectomy

はじめに

IMRTや密封小線源治療などの低侵襲で機能温存に有利な治療法が豊富に存在する現在，手術療法も腹腔鏡下前立腺全摘術（LRP），ロボット支援前立腺全摘除術（RARP），ミニマム創前立腺全摘術[1,2]など低侵襲化が進みQOLの向上に寄与している。しかしこれらの鏡視下手術のアドバンテージは低侵襲性のみならず，拡大画像による詳細な組織解剖所見を取得できることにあり[3]，より精密な手術を可能にし，機能温存のみならず根治性の向上をもたらす可能性を有しているといえる。

当センターではIMRTおよび^{125}I小線源治療を施行しており低リスク，中間リスク症例ではこれらの放射線治療を選択することが多く手術対象はリスクの高い症例が多い。われわれはHigh risk症例に対しても高い根治性を得ることを目指し手技の改良に取り組んできた。その結果小切開でも根治性の高い手術が可能と判断し2007年4月以降基本的に全例小切開腹腔鏡補助下にて行っている。われわれの行っている拡大手術の術式と治療成績を報告する。

Ⅰ．対象・方法

2007年4月1日から2009年7月28日までに行った小切開腹腔鏡補助下前立腺全摘術186例を対象とした。

1．患者背景

年齢は48～83歳，平均65.6歳であった。tPSAは3.3～65.449ng/ml，平均10.8ng/mlであった。治療前リスク分類は低リスク群，中間リスク群，高リスク群それぞれ12例(6%)，78例(42%)，96例（52%）であった。治療前のPSAは＜4ng/ml，4～10ng/ml，10～20ng/ml，20ng/ml～それぞれ10例，112例，42例，22例であった。グリソンスコアはGS4～6，GS7，GS8～10それぞれ20例，94例，71例であった。臨床病期（取り扱い規約に基づき1997年版TNMを使用）は

*栃木県宇都宮市陽南4-9-13 〒320-0834
Tel：028-658-5151 Fax：028-658-5669
受付日：2010年2月19日

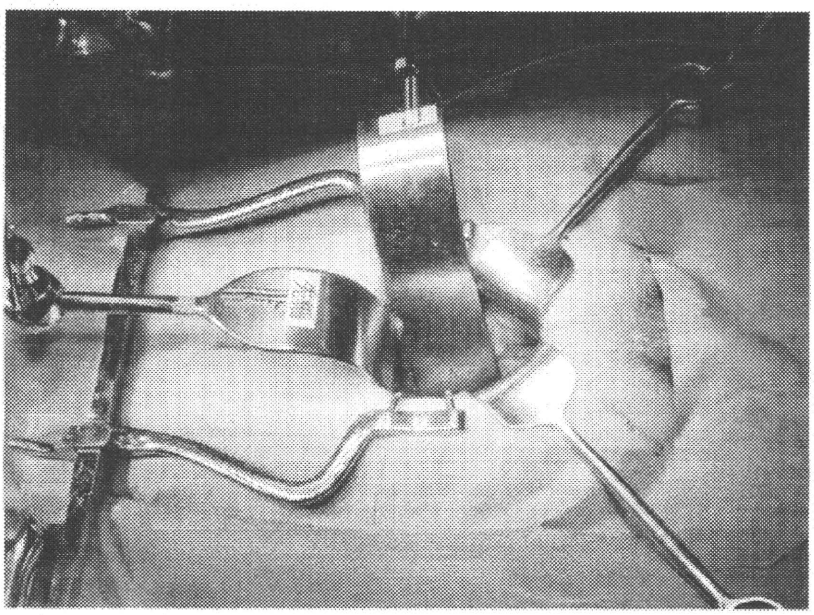

図1　オクトパス，ケント式吊り上げ鉤，小児用開創器による層の展開

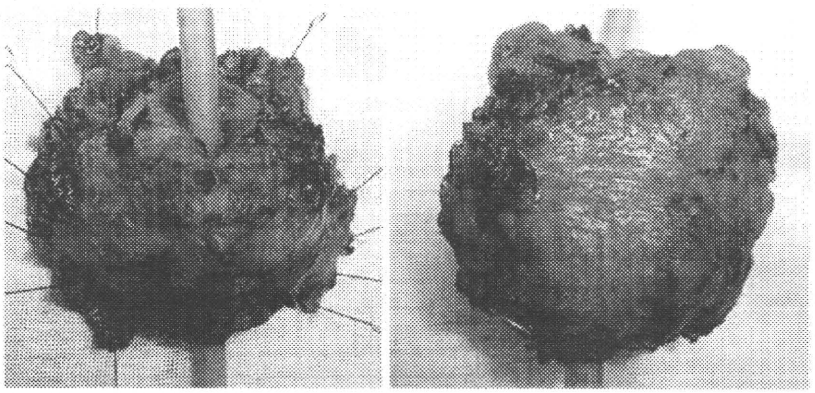

図2　摘出標本

cT1c，cT2a，cT2b，cT3a，cT3b それぞれ 49 例，63 例，32 例，38 例，1 例であった。また全例 cN0M0 であった。

2．手術手技

手術は術者と助手の 2 名で行っている。スコープホルダーを用いてスコピストは省いている。術者はヘッドランプおよび 4.5 倍または 2.5 倍のルーペを装着する。

体位は下肢を折った仰臥位。皮膚切開は 2008 年 3 月までは 7cm 台，その後は 6cm 台の下腹部正中切開。術野の展開は 2 本のオクトパスを頭側に，2 本のケント式吊り上げ鉤を尾側に配置し，小児用開創器も使用する（図 1）。オクトパスのブレードは KGS 鉤 2 の幅を狭めた特注先細鉤，KGS 鉤 3 の長さを長くし角度を浅くした特注特長鉤を使用している。オクトパスの使用により小切開でも充分に良好な視野展開が可能である。オリンパス社ハイビジョン腹腔鏡（軟性鏡）を頭側より術野に入れ全員で画像を確認し，術者も適宜観察を行う。詳細な観察が必要なときにはスコープを近接させ拡大画像を得る。

手術は plane dissection を心がけレチウス腔の展開においては脂肪組織（perivesical fat pud）[5] の除去も系統的に行い以降の操作に備える。

リンパ節郭清は中間リスク以上では特に内腸骨の内側，閉鎖の頭側端の郭清を徹底的に行う拡大リンパ節郭清を行う。

High risk 症例を完全に摘出するためには前立腺組織が一切露出しないよう enblock に摘出することが肝要である（図 2）。本術式の要点である尿道先行露出法による尖部処理，直腸縦走筋完全露出について解説する。

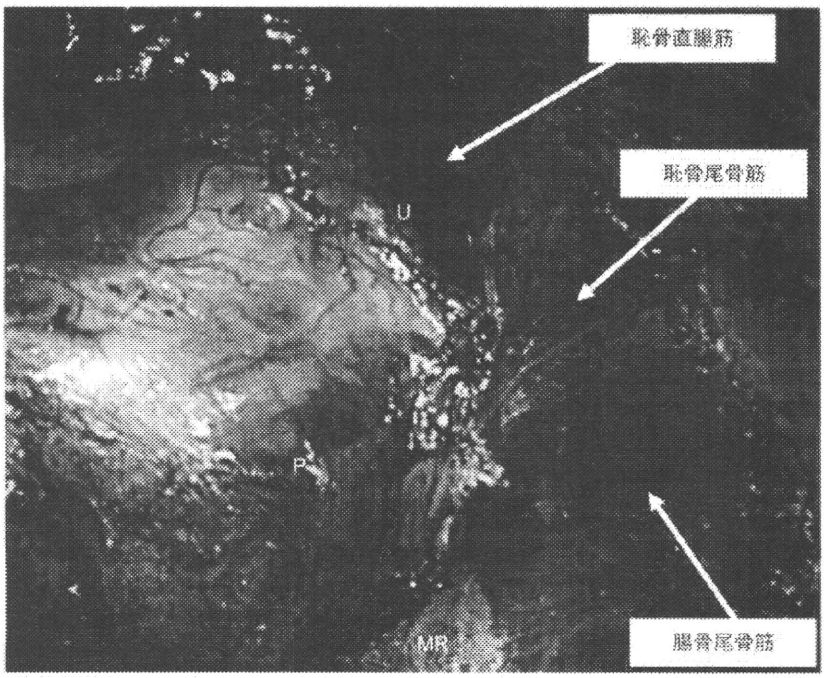

図3　前立腺の右外側の展開を斜め頭側より俯瞰した図（図の上方が恥骨側，下方が頭側）
P：前立腺，U：尿道，MR：mesorectum

3. 尿道先行露出法

肛門挙筋，肛門挙筋筋膜と前立腺尖部，尿道との解剖学的関係を意識し3つのステップよりなる操作で前立腺尖部，尿道を完全に露出する。

1）tendinous arch の直外側で下方の肛門挙筋や前立腺の静脈叢を損傷せぬよう注意しつつ肛門挙筋を切開しこれを頭尾側に広げる。露出された肛門挙筋（腸骨尾骨筋）を前立腺から鈍的に外側に圧排する（**図3**）。この操作を尖部に向かって進めるがやがて鈍的剥離が不可能になるため無理をせずここで操作を一旦中止する。

2）操作を最遠位に移し，恥骨前立腺靱帯直外側で肛門挙筋筋膜を切開し恥骨前立腺靱帯も可能な限り切離する。同部では肛門挙筋（恥骨直腸筋）と尿道との間は鈍的に剥離され（厳密にいえば尿道外側に癒合した肛門挙筋筋膜内面と肛門挙筋との間の層）（**図4**破線矢印）メッツェンバウムによる剥離で尿道後方まで容易に露出できる。

3）間に残った肛門挙筋（恥骨尾骨筋）は尖部に強固に癒合し（**図4**黒矢印）無理な剥離は出血を招くばかりでなく正しい剥離層を失うことになるのでシーリングデバイスにて切離する（**図5**）。極力筋肉を損傷しないよう注意し前立腺のシルエットに沿って切離を行う。

以上の3ステップの操作で前立腺側面～尿道が広く展開される。尿道後壁，その背側の直腸尿道筋および前立腺尖部が形成するアングルが前立腺尖部・尿道の移行部であり，ここにベッセルループを通して尿道を切離すれば例え posterior lip があっても前立腺尖部に切り込むことはない（**図6**）。

4. 直腸縦走筋完全露出（図7）

mesorectum を前立腺の十分外側で切開，直腸との間の剥離操作に移る。直腸縦走筋上の脂肪や膜（denonvillier's fascia 後葉あるいは Aigner らのいう rectogenital septum[3]）をすべからく前立腺につけ直腸縦走筋を完全に露出する層で剥離を進めると左右は貫通する。末梢は rectourethrarl-is muscle に突き当たり剥離が困難になるまで行う。直腸縦走筋を直視しながらの剥離が直腸損傷を起こさない最も安全な方法であると考える。

Ⅱ．結　果

出血量は中央値354ml（63～2,204ml）であった。自己血の準備は行っておらず同種血輸血を行った症例は1例もなかった。

合併症では直腸損傷，術後の直腸尿道瘻ともに1例も認めていない。1例で後出血のため翌日血腫除去術を施行している。

摘出標本の病理診断は pT2a，pT2b，pT3a，

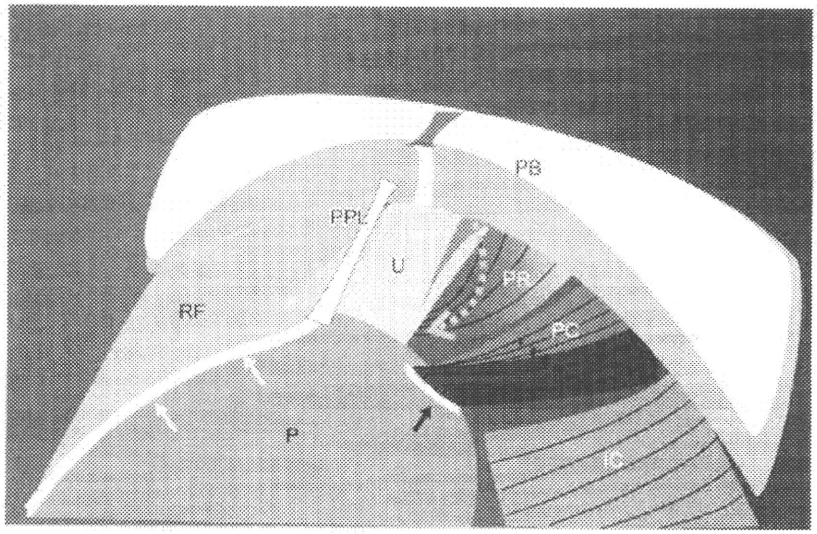

図4　図1の模式図
肛門挙筋筋膜の前立腺上での折り返しがtendinous archである（白矢印）。右側は肛門挙筋筋膜を開けた状態を図示。恥骨尾骨筋は前立腺尖部に強固に癒合する（黒矢印でポした白色部分）。Shafik ら[9]はbiatal ligamentを介して恥骨尾骨筋と尖部は連続しているとしている。尿道外側と恥骨直腸筋の間は鈍的に剥離可能である（破線矢印）。
P：前立腺，U：尿道，PB：恥骨，RF：肛門挙筋筋膜，PPL：恥骨前立腺靭帯，IC：腸骨尾骨筋，PC：恥骨尾骨筋，PR：恥骨直腸筋。

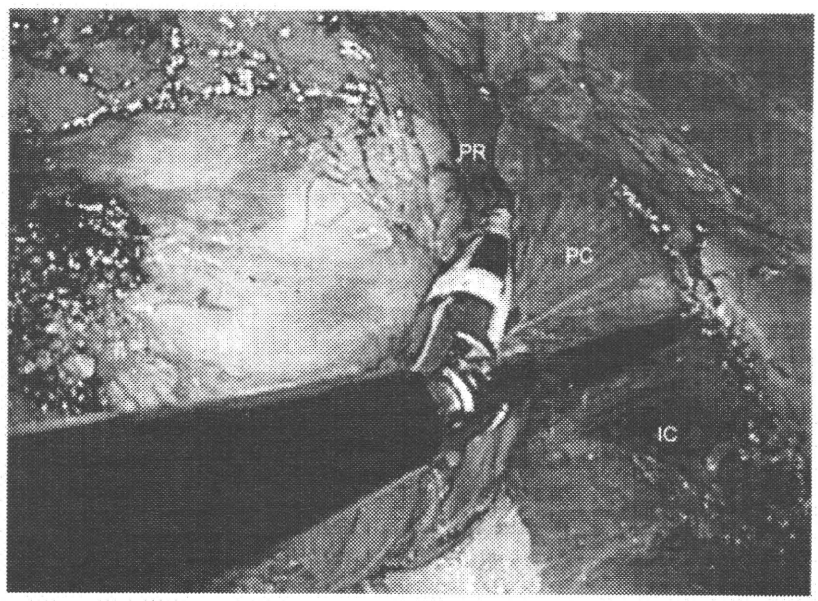

図5　恥骨尾骨筋のシーリングデバイスによる切離
IC：腸骨尾骨筋，PC：恥骨尾骨筋，PR：恥骨直腸筋

pT3b, pT4それぞれ21例(13.0%), 87例(47.0%), 58例（28.1%), 18例（9.7%), 4例（2.2%）であった。

断端陽性は168例中21例（12.9%）に認めた。pT2では断端陽性は111例中2例（1.8%）のみであった。pT3でも断端陽性は70例中19例(27.1%）であった。pT4では5例中3例（60%）であった。

Ⅲ．考　察

ミニマム創手術では腹腔鏡による拡大画像を得られ従来では認識できなかった詳細な組織構造の観察が可能である。これは肉眼手術所見と解剖所見とのギャップを補完し，詳細な組織構造の把握による操作は手術をより正確なものにし，結果根治性向上に寄与する可能性を有している。

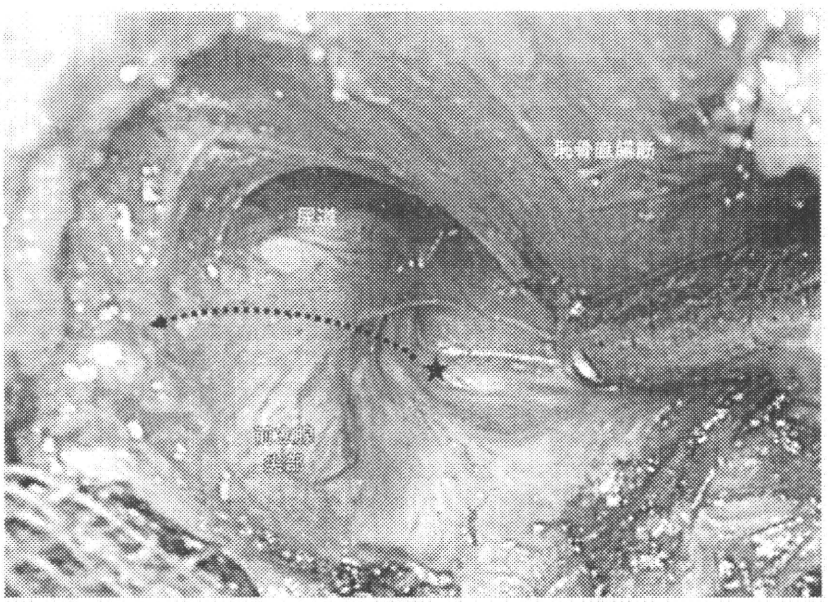

図6　尿道切断位置
尿道、前立腺尖部、直腸尿道筋とで形成するアングル（★）をメルクマールに前立腺尖部より遠位で尿道を切断（破線矢印）。

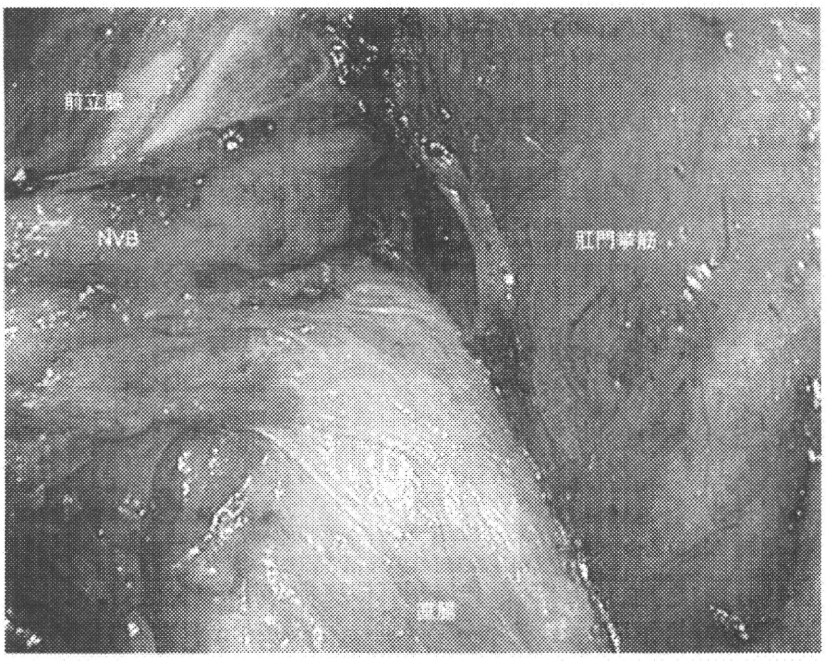

図7　直腸縦走筋完全露出

当センターでは低侵襲性の追求よりもまず根治性の向上を目指し手技の改良に取り組んできた。腹腔鏡による観察、記録は解剖の理解を飛躍的に向上させ充分根治性の高い術式（尿道先行露出、直腸縦走筋露出）に至ったが同時に創展開の工夫で小切開でも全く根治性を犠牲にせずに拡大手術が行えることが明らかになり全例ミニマム創にて手術を行に至った。ミニマム創手術では開腹手術のノウハウを活かし[6]安全に移行できるという特徴を有している。

手術成績については pT2 の断端陽性率は LRP で 4.7 ～ 17.9 %、RARP で 3.6 ～ 38.4 %、pT3 では LRP で 26.2 ～ 45.7 %、RARP で 26.3 ～ 66 % といわれている[7]。われわれの症例はリスクの高いものが多かったが（高リスクは 52%）、断端陽性は pT2 で 1.8%、pT3 でも 27.1 % と良好な成績であった。

LRP、RARP は高価なディスポーザブル製品の