厚生労働科学研究研究費補助金

がん臨床研究事業

早期前立腺がんにおける根治術後の再発に対する標準的治療法の確立に関する研究

平成18年度　総括研究報告書

主任研究者　内藤　誠二

平成19（2007）年　3月

研究報告書目次

目　　次

厚生科学研究費補助金（がん臨床研究事業）

I　総括研究報告書

主任研究者　内藤誠二

早期前立腺がんにおける根治術後の再発に対する標準的治療法の確立に関する研究

研究要旨
「早期前立腺がんにおける根治術後の再発に対する標準的治療法の確立に関する研究」（phase III 試験）の患者登録を継続している。

主任研究者氏名：　内藤誠二
所属機関名、職名：九州大学大学院医学研究院泌尿器科学分野、教授

A. 研究目的

限局性前立腺癌に対する根治的前立腺摘除術後のPSA再発患者に対して、内分泌療法前に放射線療法を行うことの臨床的有用性を、内分泌療法単独とのランダム化比較試験により評価し、標準的治療法の確立を目指すPprimary endpointは抗アンドロゲン剤（ビカルタミド）のTime-to-Treatment Failure(TTF)とし、secondary endpointはプロトコール治療のTTF、全生存期間、無増悪生存期間、放射線、抗アンドロゲン剤、LH-RHアナログの有害事象、QOLとした。

B. 研究方法

登録時に以下のA群(内分泌療法群)かB群(放射線療法群)にランダム割付される。治療開始後、臨床再発またはPSA再発を認めた場合、または有害事象や患者拒否により治療継続が困難となった場合、Time to treatment failure (TTF)と判断する。A群では抗アンドロゲン剤による治療とその後のTTFに対するLH-RHアナログ治療をもってプロトコール治療とし、B群では前立腺床に対する64.8Gyの外照射、その後TTFを生じたらA群と同様の治療をもってプロトコール治療とする。登録期間は4年、追跡期間は登録終了後5年とし、総研究期間は9年とする。

C. 研究結果

1)当研究はJapan Clinical Oncology Group(JCOG)にプロトコール審査及び、データマネージメントを依頼し研究を行なっている。H18年3月23日現在、58名の登録が得られているが、重篤な有害事象は認めていない。2) 日本における PSA 再発の状況把握のため、全参加施設を対象に過去 5 年間の PSA 再発患者、1192 名の臨床データを後ろ向きに調査、解析し、その結果を BJU Int., 98:549-553,2006 に報告した。3) H18 年度は、患者登録推進のため、膀胱癌研究 JCOG0209 のみに参加していた施設を本研究への参加施設として新たに加えた。また、2 施設を inactive とみなし、新規施設との入れ替えを行った。これらの新規参加施設ではすでに IRB の承認を得て、登録可能な状況となっている。4) H18 年度末の調査で、まだ登録可能な PSA のレベルにまでは達していないものの、確実に術後 PSA の再上昇をきたしている登録候補患者が全参加施設ですでに 259 名いることを確認した。これらの患者を確実に recruit することで登録が推進されるものと期待される

D. 考察

登録患者数が予定を下回っており、登録を増やすための種々の工夫を行なっている。今後も積極的に患者登録を進めて行きたい。

E. 結論

現在、患者登録を行い、プロトコール治療を推進中である。現在までに治療による重篤な有害事象は認めていない。

F. 健康危険情報

なし。

G. 研究発表

1. 論文発表

Yokomizo A, Murai M, Baba S, Ogawa O, Tsukamoto T, Niwakawa M, Tobisu K, Kinukawa N, Naito S. Percentage of positive biopsy cores, preoperative prostate-specific antigen (PSA) level, pT and Gleason score as predictors of PSA recurrence after radical prostatectomy: a multi-institutional outcome study in Japan. BJU Int., 98:549-553, 2006.

2. 学会発表 なし。

H. 知的財産権の出願・登録状況

1. 特許取得 なし
2. 実用新案登録　なし。

II-1 分担研究報告書

分担研究者　塚本 泰司

前立腺癌の発生と進展に関する疫学、遺伝子解析研究

研究要旨
前立腺癌における神経内分泌細胞と前立腺癌の進展に関する基礎的検討を行った。

分担研究者氏名：　塚本泰司
所属機関名、職名：札幌医科大学泌尿器科　教授

A. 研究目的
前立腺癌において神経内分泌(NE)細胞が周囲に存在する前立腺癌細胞の増殖あるいは進展に関与する可能性が指摘されているが、結論は出ていない。神経内分泌細胞癌から成るトランスジェニックマウス前立腺癌（NE-10）とその継代樹立細胞を用いて、この細胞がヒト前立腺癌培養細胞(LNCaP)の増殖、浸潤能、転移能に与える影響を検討した。

B. 研究方法ヌードマウスのそれぞれの側の背部皮下にNE-10細胞とLNCaP細胞を移植し、各々の細胞株の増殖能、浸潤能に与える影響を検討した。

C. 研究結果
LNCaP細胞の増殖はNE-10細胞によって影響されなかった。しかし、NE-10細胞の移植を行った場合とそれを行わなかった場合とを比較すると、前者の場合には対側に移植したLNCaP細胞の肺転移を有意に増強した。さらに、NE-10細胞の培養上清はLNCaP細胞の遊走能、浸潤能を明に促進した。DNA microarrayによる分析では、培養上清で処理したLNCaP細胞の8つのmRNAの発現増強が確認された。このうち、ゲルゾリンのmRNAに注目してその経時的な発現をNorthern blotで確認すると、4、8時間ともその発現が対照と比較して明らかに増強されていた。

D. 考察
NE-10細胞から分布されるゲルゾリンなどの物質によりLNCaP細胞の浸潤能が増強され、肺転移が促進されたと考えられた。

E. 結論
前立腺癌において神経内分泌(NE)細胞が周囲に存在する前立腺癌細胞の増殖あるいは進展に関与する可能性が示唆された。

G．研究発表
1.　論文発表
1. Uchida K, Masumori N, Takahashi A, Itoh N, Kato K, Matsusik RJ, Tsukamoto T. Murine androgen-independent neuroendocrine carcinoma promotes metastasis of human prostate cancer cell line LNCaP. Prostate, 66: 536-545, 2006.

2. Fukuta F, Hisasue S, Yanase M, Kobayashi K, Miyamoto S, Kato S, Shima M, Tsukamoto T, Takatsuka K. Preoperative computed tomography finding predicts for postoperative inguinal hernia: new perspective for radical prostatectomy-related inguinal hernia. Urology, 68: 267-271, 2006.

3. Kitamura H, Torigoe T, Asanuma H, Hisasue S, Suzuki K, Tsukamoto T, Sato M, Sato N. Cytosolic overexpression of P62 sequestosome 1 in neoplastic prostatic tissue. Histopathology, 48:157-161, 2006.

2.　学会発表

H. 知的財産権の出願・登録状況
1. 特許取得 なし
2. 実用新案登録　なし。

厚生科学研究費補助金（がん臨床研究事業）

II-2 分担研究報告書

分担研究者　村井　勝

早期前立腺癌に対する各種根治療法の臨床病理的検討

研究要旨
根治的前立腺摘除術が施行された症例において、最大腫瘍径は癌の浸潤進展度に関与する重要な病理組織学的因子である

分担研究者氏名：　村井　勝
所属機関名、職名：慶應義塾大学医学部泌尿器科教授

A. 研究目的
根治的前立腺摘除術が施行された症例において、前立腺癌の局所浸潤進展度と腫瘍の大きさを含めた病理組織学的因子との関連性を検討した。

B. 研究方法
術前に限局性前立腺癌と診断され、内分泌療法や放射線療法を行わず、根治的前立腺摘除術が施行された 164 症例を対象とした。それぞれの症例の病理組織標本において、神経繊維周囲浸潤の有無、脈管浸潤の有無、被膜外進展の有無、精嚢浸潤の有無といった病理組織学的浸潤度、Gleason score による分化度、各プレパラート切片の最大腫瘍面積、最大腫瘍径および全腫瘍体積を求め、局所浸潤進展度に関連する病理組織学的因子を検討した。

C. 研究結果
病理学的病期(pT2 群と pT3 群)と脈管浸潤の有無、神経繊維周囲浸潤の有無、精嚢浸潤の有無ならびに Gleason score は有意な関連性を示した。また、pT2 群の全腫瘍体積、最大腫瘍面積、最大腫瘍径はそれぞれ 2.88 ± 0.32 cm^3、1.41 ± 0.12 cm^2、1.78 ± 0.08 cm であり、pT3 群のそれら（5.87 ± 0.69 cm^3、2.50 ± 0.22 cm^2、2.52 ± 0.10 cm）に比べて有意に小さかった。ロジスティック回帰分析では、局所浸潤進展度に関与する有意な病理組織学的因子は Gleason score、脈管浸潤の有無、神経繊維周囲浸潤の有無と最大腫瘍径であった。

D. 考察
限局性前立腺癌において、最大腫瘍径はGleason score、脈管浸潤の有無、神経線維周囲浸潤とともに癌の浸潤進展度に関与する病理組織学的因子であることが示された。

E. 結論
最大腫瘍径は癌の浸潤進展度に関与する重要な病理組織学的因子である。

G．研究発表

1.　論文発表

1. Mizuno R, Nakashima J, Mukai M, Ookita H, Nakagawa K, Oya M, Ohigashi T, Marumo K, Murai M. Maximum tumor diameter is a simple and valuable index associated with the local extent of disease in clinically localized prostate cancer. Int. J. Urol., 13: 951-955, 2006.

2. Kikuchi E, Nakashima J, Ishibashi M, Ohigashi T, Oya M, Nakagawa K, Miyajima A, Murai M. Usefulness of alpha (1) -antichymotrypsin-PSA complex for predicting bone metastases of prostate cancer. Urology, 68:371-375, 2006.

3. Kosaka T, Miyajima A, Takayama E, Kikuchi E, Nakashima J, Ohigashi T, Asano T, Sakamoto M, Okita H, Murai M, Hayakawa M. Angiotensin II type 1 receptor antagonist as an angiogenic inhibitor in prostate cancer. Prostate, 67:41-49, 2007.

4. Kuroda K, Nakashima J, Kanao K, Ekikuchi E, Miyajima A, Horiguchi Y, Nakagawa K, Oya M, Ohigashi T, Murai M. Interleukin 6 is associated with cachexia in patients with prostate cancer. Urology, 69:113-117, 2007.

2.　学会発表

H. 知的財産権の出願・登録状況
1. 特許取得 なし
2. 実用新案登録　なし。

厚生科学研究費補助金（がん臨床研究事業）

II-3 分担研究報告書

分担研究者　井川幹夫

早期前立腺癌に対する各種根治療法の臨床病理的検討

研究要旨
High-risk 前立腺癌に対する術前 8 ヶ月の補助内分泌療法の有用性に関する検討を行った。

分担研究者氏名：　井川幹夫
所属機関名、職名：島根大学医学部泌尿器科
教授

A. 研究目的

転移性前立腺癌に対するタキサン系抗癌剤（Taxane/パクリタキセル（PTX）およびドセタキセル（DTX））にエストラムスチン（EMP）とカルボプラチン（CBDCA）を併用する癌化学療法（TEC 療法/PEC および DEC 療法）の基礎的根拠を high-mobility group 1（HMG1）蛋白（DNA 修復蛋白質の結合阻害）の発現から示し、実際の TEC 療法の有効性と安全性を評価し、PEC 療法と DEC 療法を比較した。また、ホルモン抵抗能獲得への関与が示唆され、タキサン系抗癌剤によりその機能が抑制される Bcl-2 蛋白（抗アポトーシス）発現と TEC 療法による治療効果との関係も評価した。。

B. 研究方法

前立腺癌細胞株に対して、エストロゲン剤と白金製剤（PTX および DTX）を投与し細胞増殖抑制効果、アポトーシスの出現頻度、HMG1 蛋白発現量を観察した。 また、HRPC に対する TEC 療法を 81 例（PEC 療法：30 例、DEC 療法：51 例）に施行した。治療は PTX 100 mg/m^2 or DTX 30 mg/m^2/週 1 回静注、EMP 10 mg/kg/day/連日経口投与、CBDCA AUC=6/4 週毎静注を 1 サイクルとし、重篤な有害事象あるいは Progression がみられるまで継続した。また Bcl-2 蛋白およびそのファミリー蛋白（Bak, Bax）発現レベルを免疫組織化学的に検討し、TEC 療法の治療効果との相関も調べた。

C. 研究結果

前立腺癌細胞において、細胞増殖抑制効果およびアポトーシス出現頻度は、白金製剤単独投与群に比べてエストロゲン剤と白金製剤の併用投与群では有意に増加した。 また HMG1 蛋白発現も有意に上昇した。TEC（PEC, DEC）療法における血清 PSA 値低下率が 50%および 90%以上であった症例は 97.5（100, 96.1）%、67.9（63.3, 70.6）%であった。血清 PSA 値低下率、Time to progression および Overall survival time (OST) の中央値は、それぞれ 94.7 (91.3, 96.0) %、11.3 (10.2, 12.2) ヶ月および 26.6 (20.5, 33.2) ヶ月で、DEC 療法のほうが PEC 療法に比べ有意に優れた治療効果を示した。測定可能病変および骨転移巣に対する奏効率は、両者に有意差はなかった。いずれにも治療関連死はなく、骨髄毒性、肝機能異常、浮腫、食欲不振、疲労を両群で比較的多く認めたが差はなかった。PEC 療法では重篤な末梢神経障害、DEC 療法では流涙、爪の変化を多く認めた。また、化学療法前 Bcl-2 陽性症例は陰性症例と比較し TEC 療法での OST の延長が認められ、多変量解析でも独立因子となった。

D. 考察

TEC療法、特にDTXを用いたDEC療法は優れた治療効果を示した。有害事象の発生頻度は比較的高く、今後さらなる治療法の開発が望まれる。また、TEC 療法前Bcl-2蛋白発現量はTEC療法を選択する際の治療効果を予見する有用な指標となり得える。

E. 結論=転移性前立腺癌に対するタキサン系抗癌剤を中心とした癌化学療法にEMP とCBDCAを併用するTEC療法の根拠が示された。

G．研究発表

1. 論文発表

Kikuno N, Urakami S, Nakamura S, Hiraoka T, Hyuga T, Arichi N, Wake K, Sumura M, Yoneda T, Kishi H, Shigeno K, Shiina H, Igawa M. Phase-II Study of Docetaxel, Estramustine Phosphate, and Carboplatin in Patients with Hormone-Refractory Prostate Cancer. Eur Urol., in press, 2006,

2. 学会発表

H. 知的財産権の出願・登録状況

1. 特許取得 なし
2. 実用新案登録　なし

II-4 分担研究報告書

分担研究者　馬場　志郎

早期前立腺癌に対する各種根治療法の臨床病理的検討

研究要旨
High-risk 前立腺癌に対する術前 8 ヶ月の補助内分泌療法の有用性に関する検討を行った。

分担研究者氏名：　馬場　志郎
所属機関名、職名：北里大学医学部泌尿器科
教授

A. 研究目的
High-risk 前立腺癌における根治的前立腺全摘除術の治療成績は、その高い PSA 再発率が問題となっており、このような問題を改善すべく、完全切除の可能性を高くする目的で術前内分泌療法が施行されているものの、その有効性については未だ結論が得られていない。今回、その臨床的意義について検討した。

B. 研究方法
多施設共同前向き研究として、2000 年 7 月から 2003 年 5 月までに、当院および関連施設で high-risk 前立腺癌（臨床病期 >T2c:1992 TNM、または PSA 値＞20.0 ng/ml、または Gleason score >8）と診断され、術前 8 ヶ月 NHT 後、根治的前立腺全摘除術を施行した 21 例を対象とした。

C. 研究結果
前立腺生検前 PSA 中央値は 27.6 ng/ml（8.5-80.7 ng/ml）、術前 PSA 中央値は 0.28 ng/ml（0.02-4.2 ng/ml）であり、術前 8 ヶ月 NHT 後、血清 PSA が測定感度(0.02ng/ml)以下に達した症例を 5 例（23.8%）に認めた。T 分類では T1c 9 例、T2a-b 8 例、T2c 3 例、T3a 1 例で、術後観察期間は 4-37 ヶ月（中央値 25 ヶ月）であった。摘出前立腺標本における外科的切除縁陽性症例を 5 例(23.8%)、被膜外浸潤を 5 例（23.8%）、pT0 を 2 例(9.5%)に認めた。生物学的再発は 9 例（42.9%）に認められ、うち 1 例は pT0 症例であった。術後生物学的再発までの期間は 2-25 ヶ月（中央値 6 ヶ月）で、1 例を除き 12 ヶ月以内に生じた。生物学的再発率は、精嚢浸潤症例において有意に (p=0.0308) 高く、術前 8 ヶ月 NHT 後の PSA 値が、0.1ng/ml 以上の症例に高い傾向（p=0.0836）を認めた。

D. 考察
High-risk前立腺癌に対する術前8ヶ月NHTを行ったが、その多くが早期にPSA再発を認める結果であった。またmaximum tumor damage を呈しているpT0症例においても、早期にPSA再発していることから、いわゆるtumor eliminationには至っていないと考えられ、術前8ヶ月NHTに対する有用性は見出せなかった。

E. 結論=今回の検討ではHigh-risk前立腺癌に対する術前8ヶ月NHTに対する有用性は見出せなかった。

G．研究発表

1. 論文発表
1. Uchida T, Ohkusa H, Nagata Y, Hyodo T, Satoh T, Irie A. Treatment of localized prostate cancer using high-intensity focused ultrasound. BJU Int., 97:56-61, 2006.
2. Fujita T, Teh BS, Timme TL, Mai WY, Satoh T, Kusaka N, Naruishi K, Fattah EA, Aguilar-Cordova E, Butler EB, Thompson TC. Sustained long-term immune responses following *in situ* gene therapy combined with radiotherapy and hormonal therapy in prostate cancer patients. Int J Radiat Oncol Biol Phys 65: 84-90, 2006.
3. Ishiyama H, Kitano M, Satoh T, Niibe Y, Uemae M, Fujita T, Baba S, Hayakawa K. Difference in rectal dosimetry between pre-plan and post-implant analysis in transperineal interstitial brachytherapy for prostate cancer. Radiother Oncol. 78:194-198, 2006.
2. 学会発表

H. 知的財産権の出願・登録状況
1. 特許取得 なし
2. 実用新案登録　なし。

II-5 分担研究報告書

分担研究者　中川　昌之

早期前立腺癌に対する根治術後のQOLの解析と新たなbiomarkerの開発に関する研究

研究要旨
ヒト前立腺癌の診断および術前ステージ予測のための新しいバイオマーカー：Multi-gene methylation analysis score (M-score)の有用性の検討。

分担研究者氏名：　中川　昌之
所属機関名、職名：鹿児島大学医学部泌尿器科学教授

A. 研究目的
ヒト前立腺癌（PC）では様々な遺伝子のプロモーターのメチル化が報告されている。我々は複数の遺伝子のメチル化を測定しスコア化(M-score)して、PC の診断および術前のステージ予測マーカーとしての有用性を検討した。

B. 研究方法
前立腺全摘術 PC170 例および TUR-P 施行の前立腺肥大症(BPH)69 例を対象とした。3 つの遺伝子（APC、GSTP1、MDR1）のメチル化は methylation specific PCR 法により評価した。さらに多変量 logistic 回帰分析により得られた各遺伝子の log hazard ratio を合計することにより、M-score を算出した。

C. 研究結果
M-scoreは有意にpT分類（$p<0.001$）、Gleason score（$p<0.001$）、術前PSA値（$p<0.027$）と関係していた。全患者においてM-score は診断マーカーとして有用であった(カットオフ1.0の場合、感度75.9%、特異度84.1%)。全PC患者においてM-scoreは限局性PC(≦pT2)と局所進行PC(≧pT3)を区別する術前のステージ予測マーカーとして有用であった(カットオフ4.0の場合、感度72.1%、特異度67.8%)。さらに術前PSA値が10.0 ng/ml未満のPC患者104人においてはM-scoreはPSAよりも術前のステージ予測マーカーとして有用であった($p<0.010$)。

D. 考察
M-scoreはPCの診断および術前のステージ予測マーカーとして有用であった。特にlow PSAの症例においてPSAよりも優れたステージ予測マーカーであることが示唆された。

E. 結論= M-scoreは優れた腫瘍マーカーであり、特にlow PSAの症例においてPSAよりも優れたステージ予測マーカーであることが示唆された。

G．研究発表

1. 論文発表
Enokida H, Shiina H, Urakami S, Terashima M, Ogishima T, Li LC, Kawahara M, Nakagawa M, Kane CJ, Carroll PR, Igawa M, Dahiya R. : Smoking influences aberrant CpG hypermethylation of multiple genes in human prostate carcinoma. Cancer, 106:79-86, 2006.

2. 学会発表
第94回　日本泌尿器科学会総会（福岡）

H. 知的財産権の出願・登録状況
1. 特許取得 なし
2. 実用新案登録　なし。

II-6 分担研究報告書

分担研究者　筧　善行

早期前立腺癌に対する根治術後のQOLの解析と新たなbiomarkerの開発に関する研究

研究要旨
根治術後の QOL の解析を行なうとともに、新たな biomarker の開発を行なっている。

分担研究者氏名：　筧　善行
所属機関名、職名：香川大学医学部泌尿器科
教授

1）日本人限局性前立腺癌患者のための新しい疾患特異的 QOL 調査票の作成

限局性前立腺癌患者の QOL 評価票として UCLA-Prostate Cancer Index（以下 UCLA-PCI）が頻用されてきたが、近年普及しつつある小線源治療施行患者や、様々な局面で施行されるホルモン療法にも対応した QOL 評価票として、UCLA-PCI を発展させた EPIC 調査票が米国で開発された。我々は EPIC の日本語版を NPO 法人健康医療評価研究機構 I-HOPE と共同で作成し、全国 10 施設 460 名の患者を用いて妥当性・信頼性を検証した（J Urol 2007 年 5 月号印刷中）。

さらに、UCLA-PCI はすでに我が国において、前立腺癌に関する大規模な臨床研究（JCOG0401 を含む）で使用されているが、これらで得たデータを EPIC を使用した解析結果と比較するための相関性に関する検証も行った（J Urol 2007 年 8 月号印刷中）。

2）Death receptor 4 および 5 に対する特異的ヒト型抗体による前立腺癌に対する新規治療法の開発

Death receptor 4 および 5 に対する特異的ヒト型抗体 HGS-ETR1, ETR2 は TRAIL を投与したのと同じように Death receptor を介して癌細胞特異的にアポトーシスを誘導する。TRAIL の投与よりも decoy receptor による干渉を受けず、効率的かつ経済的に制がん効果を期待できる長所がある。今回、ホルモン非感受性ヒト前立腺癌細胞においても、DR4 および 5 の抗腫瘍活性が確認できた（Urology 印刷中）。さらに低容量のアドリアマイシンが DR-4 を介した抗腫瘍効果を増強することもつきとめた（J Urol 印刷中）。

G．研究発表

1. 論文発表

1）Kakehi Y, Takegami M, Suzukamo Y, et al. Health-related QOL of Japanese men with localized prostate cancer treated with current multiple modalities assessed by a newly developed Japanese versio of EPIC (the Expanded Prostate Cancer Index Composite). J Urol (in press)

2）Shimada O, Wu X, Kakehi Y, et al. Human agonistic antibody to tumor necrosis factor-related apotosis-inducing ligand receptor 2 (Trail-R2) induces cytotoxicity and apoptosis in prostatic cancer and bladder cancer cells. Urology (in press)

3）Namiki S, Arai Y, Kakehi Y, et al. Analysis linking UCLA PCI with EPPIC: an evaluation health-related QOL of Japanese men with localized prostate cancer. J Urol (in press)

2. 学会発表

H. 知的財産権の出願・登録状況

1. 特許取得 なし
2. 実用新案登録　なし。

III 研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yokomizo A, Murai M, Baba S, et al.	Percentage of positive biopsy cores, preoperative prostate-specific antigen (PSA) level, pT and Gleason score as predictors of PSA recurrence after radical prostatectomy: a multi-institutional outcome study in Japan	BJU Int.	98	549-553	2006
Uchida K, Masumori N, Takahashi, A et al.	Murine androgen-independent neuroendocrine carcinoma promotes metastasis of human prostate carcer cell line LNCaP.	Prostate	66	536-545	2006
Mizuno R, Nakashima J, Mukai M, et al.	Maximum tumor diameter is a simple and valuable index associated with the local extent of disease in clinically localized prostate cancer.	Int. J. Urol.	13	951-955	2006
Uchida T, Ohkusa H, Nagata Y, et al.	Treatment of localized prostate cancer using high-intensity focused ultrasound.	BJU Int.	97	51-61	2006
Enokida H, Shiina H, Urakami S, et al.	Smoking influences aberrant CpG hypermethylation of multiple genes in human prostate carcinoma.	Cancer	106	79-86	2006
Kikuno N, Urakami S, Nakamura S, et al.	Phase-II Study of Docetaxel, Estramustine Phosphate, and Carboplatin in Patients with Hormone-Refractory Prostate Cancer.	Eur Urol.		In press	2006
Nasu Y, Saika T, Ebara S, et al.	Suicide Gene Therapy With Adenoviral Delivery of HSV-tK Gene for Patients With Local Recurrence of Prostate Cancer After Hormonal Therapy	Mol. Ther	15	843-840	2007
Yamauchi A, Kawai K, Tsukamoto S et al.	Persistence of prostatic intraepithelial neoplasia after effective chemoprevention of microscopic prostate cancer with antiandrogen in a rat model.	J Urol	175	348-352	2006

Okugi H, Nakazato H, Matsui H., et al.	Association of the polymorphisms of genes involved in androgen metabolism and signaling pathways with familial prostate cancer risk in a Japanese population	Cancer Detect. Prev.	30	262-268	2006
Miyake H, Kurahashi T, Hara I et al.	Significance of micrometastases in pelvic lymph nodes detected by real-time reverse transcriptase polymerase chain reaction in patients with clinically localized prostate cancer undergoing radical prostatectomy after neoadjuvant hormonal therapy	BJU Int.	99	315-320	2006
Miyake H, Hara I, Kurahashi T, et al.	Quantitative detection of micrometastases in pelvic lymph nodes in patients with clinically localized prostate cancer by real-time reverse transcriptase-PCR.	Clin. Cancer Res.	13	1192-1197	2007

Ⅳ．研究成果の刊行物・別刷

Percentage of positive biopsy cores, preoperative prostate-specific antigen (PSA) level, pT and Gleason score as predictors of PSA recurrence after radical prostatectomy: a multi-institutional outcome study in Japan

AKIRA YOKOMIZO[1], MASARU MURAI[2], SHIRO BABA[3], OSAMU OGAWA[4], TAIJI TSUKAMOTO[5], MASASHI NIWAKAWA[6], KEN-ICHI TOBISU[6], NAOKO KINUKAWA[7] and SEIJI NAITO[1]

[1]Departments of Urology and [7]Medical Information Sciences, Graduate School of Medical Science, Kyushu University, Fukuoka, Departments of Urology, [2]Keio University School of Medicine, Tokyo, [3]Kitasato University School of Medicine, Sagamihara, [4]Kyoto University Graduate School of Medicine, Kyoto, [5]Sapporo Medical University, Sapporo and [6]Shizuoka Cancer Center Hospital, Shizuoka, Japan

Accepted for publication 5 May 2006

OBJECTIVE

To evaluate the clinical outcome of radical prostatectomy (RP) in Japan, by retrospectively analysing the clinicopathological data in patients with clinical T1-T2 prostate cancer treated by RP, as there can be prostate-specific antigen (PSA) recurrence after RP in substantially many patients, and its character can differ according to ethnic group and/or country.

PATIENTS AND METHODS

We reviewed 1192 patients who had a RP from 1993 to 2002 with no neoadjuvant/adjuvant therapy and whose PSA level after RP decreased at least once to undetectable levels (<0.2 ng/mL). PSA recurrence was defined as ≥0.20 ng/mL. The patient data were collected from the Urological Oncology Study Group, a subgroup of Japan Clinical Oncology Group.

RESULTS

The patients' median (range) age was 67 (47-83) years and their PSA level before RP was 8.7 (1.0-153) ng/mL. During the median follow-up of 45.6 months, 302 of the 1192 patients (25.3%) developed PSA recurrence. The median time to recurrence was 369 (61-2128) days after RP. A log-rank test showed that five significant clinicopathological factors were associated with PSA recurrence after RP: The percentage of prostate needle-biopsy cores with cancer, the biopsy Gleason score, PSA level before RP, pathological stage, and the Gleason score of the RP specimen ($P < 0.001$ for all). In multivariate analyses, the percentage of positive biopsy cores, PSA level before RP, pT and the Gleason score of the RP specimen were all independent significant predictors of PSA recurrence after RP in Japanese men.

CONCLUSIONS

The frequency of PSA recurrence after RP was 25.3% in Japan and the percentage of positive biopsy cores, PSA level before RP, pT and the Gleason score of the RP specimen were independent significant factors for PSA recurrence.

KEYWORDS

prostate cancer, PSA recurrence, radical prostatectomy

INTRODUCTION

Prostate cancer is one of the most common malignancies among men in western countries [1]. Radical prostatectomy (RP) has been established as one of the standard management options for localized prostate cancer [2], but the outcome of RP might differ among countries due to racial, economic and medical factors. Very few outcome studies have so far been reported on RP in Japan, and therefore a large-scale investigation was planned to reveal the characteristics of PSA recurrence after RP in Japan, associated with a prospective randomized controlled trial after RP [3].

PATIENTS AND METHODS

We reviewed 1192 patients who had a RP from 1993 to 2002 with no neoadjuvant/adjuvant therapy and whose PSA level after RP decreased at least once to undetectable levels (<0.2 ng/mL), suggesting that the surgical resection was complete biochemically. All the patients enrolled were Japanese and the RP was done at 37 specialized institutes belonging to Urologic Oncology Study Group in the Japan Clinical Oncology Group (Appendix). In this group, a randomized controlled trial was started to evaluate radiotherapy and endocrine therapy for PSA failure after RP [3]. Apart from this study, the patients' data were collected and analysed to design an appropriate randomized trial. PSA recurrence was defined as a PSA level of ≥0.2 ng/mL after decreasing to an undetectable level (<0.2 ng/mL) after RP. Information was recorded on patient age, the positive number/total number of biopsy specimens, biopsy Gleason score (GS), clinical stage, PSA level before RP, the date of RP, the operative procedure (open or laparoscopic), pT, pN, GS of the RP specimen, PSA recurrence or not, the date of PSA recurrence, the timing and type of secondary cancer treatment after PSA recurrence, clinical recurrence or not, the latest follow-up date and the date of death. The margin status of the RP specimen, i.e. the external wedge, was not investigated in this

TABLE 1 Descriptive characteristics of the 1192 patients who had a RP

Characteristic	Value
Median (range):	
Age at RP, years	67 (48–83)
PSA before RP, ng/mL	8.7 (0.8–153)
Biopsy GS	
Median GS	6
N (%):	
4	234 (19.6)
5–6	441 (37.0)
7	260 (21.8)
8–10	127 (10.7)
unknown	130 (10.9)
Clinical stage	
T1	27 (2.3)
T1c	626 (52.5)
T2a	378 (31.7)
T2b	149 (12.5)
unknown	12 (1.0)
Pathological GS score of RP specimen	
Median GS	7
N (%):	
4	81 (6.3)
5–6	449 (37.7)
7	478 (40.1)
8–10	128 (10.7)
unknown	56 (4.7)
pT stage, n (%)	
T0	6 (0.5)
T2	778 (65.3)
T3	389 (32.6)
T4	2 (0.2)
TX	17 (1.4)
Positive lymph nodes, n (%)	21 (1.8)

TABLE 2 Results from the univariate analysis of clinicopathological factors

Variable	P
Biopsy GS	<0.001
PSA level before RP	<0.001
pT stage	<0.001
GS of RP specimen	<0.001
Percentage of positive biopsy cores	<0.001
Age	0.350
pN	0.960
RP method (laparoscopic or open)	0.567

study because not all the institutes prepared whole mounts of the RP specimens. The results were assessed statistically using the Kaplan–Meier method, univariate log-rank tests and a multivariate analysis using Cox's proportional hazards.

TABLE 3 Results from the multivariate survival analysis with the Cox proportional hazards model, based on the biopsy GS, PSA level before RP, pT stage, RP specimen GS, percentage of positive biopsy cores and biochemical recurrence

Variable	Hazard ratio (95% CI)	P
Biopsy GS (≤6 vs ≥7)	–	0.1301
PSA level before RP (< vs ≥ 10)	1.84 (1.40–2.40)	<0.001
pT (≤2 vs ≥3)	1.77 (1.35–2.32)	<0.001
GS of RP (≤7 vs ≥8)	1.81 (1.29–2.53)	0.001
% +ve biopsy cores (< vs ≥60)	2.05 (1.46–2.86)	<0.001

RESULTS

In all, 1192 patients were included in the analysis; their characteristics are shown in Table 1; comparing the clinical stage and RP pT stage suggested that almost a third of patients were understaged before RP (Table 1). After a median (range) follow-up of 45.6 (1.8–132.6) months, 302 patients (25.3%) had a PSA recurrence; the median time to PSA recurrence after RP was 369 (177–3977) days.

The log-rank test showed that five significant clinicopathological factors were associated with PSA recurrence after RP, i.e. biopsy GS (threshold 7, Fig. 1a), the PSA level before RP (10 ng/mL, Fig. 1b), the pathological stage (pT3, Fig. 1c), the GS of the RP specimen (8, Fig. 1d) and the percentage of positive biopsy cores (60%, Fig. 1e; all $P < 0.001$). In a univariate analysis, these five factors were also significant prognostic variables for PSA recurrence after RP in Japan (Table 2). However, age, pN, RP method (laparoscopic or open surgery) were not significantly associated with PSA recurrence (Table 2). A multivariate survival analysis with Cox's proportional regression indicated that the PSA level before RP, pathological stage, GS of the RP specimen, and the percentage of positive biopsy cores were all powerful independent predictors of PSA recurrence (Table 3). The biopsy GS was a significant factor on the univariate analysis but not on the multivariate analysis (Table 2).

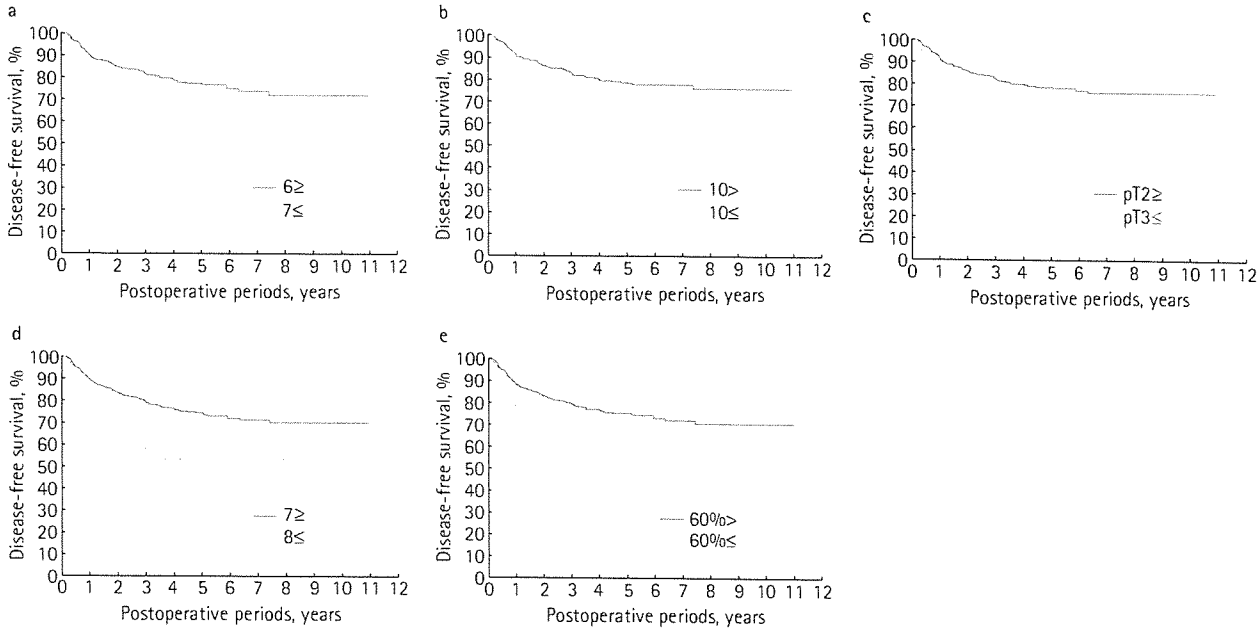
The year that the RP was done might influence the outcome, because operative skill can improve with time and the indication for RP might change depending on the year. Therefore, PSA failure-free survival rate (Kaplan–Meier) in each year was calculated, but there was no significant difference (data not shown). In addition, we did not investigate the number of patients who were lost to follow-up, but if considering patients who did not visit the hospital for ≥1 year as lost to follow-up, there were 20 (22.2%) in 1993–95, 81 (27.0%) in 1996–98 and 92 (11.5%) in 1999–2002. However, for the survival outcome, only seven patients died from cancer-related causes (data not shown), thus suggesting that the survival outcome was quite good with this treatment.

DISCUSSION

Patients enrolled in the present study represent the total experience of many surgeons from various geographical locations, and of different pathologists and their techniques, in Japan. Indeed, this study was retrospective and multi-institutional, and therefore the data described do not necessarily represent the real data on RP in Japan. Furthermore, the pathological results of the biopsy and RP specimen were not derived from central pathologists, and therefore might contain errors in GS and pT diagnosis. However, we think that this study reflects the Japanese urological community as a whole, avoiding any institutional bias and not favouring any one technique.

Pathological and epidemiological data suggest that racial variation exists for the clinically diagnosed form of prostate cancer, i.e. it is highest in African-Americans, next highest in Caucasians and lowest in Asians [4], thus suggesting that the bioactivity of prostate cancer is different for each ethnic group. However, very few outcome results of large-scale studies have been reported on RP in Japan, and thus the present study was planned to clarify the outcome of Japanese patients treated by RP. An important point of

FIG. 1. Actuarial 10-year Kaplan–Meier estimates of biochemical recurrence rates in patients who had RP, stratified according to: ***a****, biopsy GS; the PSA RFS at 5 years was 77.3% in those with GS ≤6 and 66.2% for GS ≥7.* ***b****, the PSA level before RP; the PSA RFS at 5 years was 79.2% for PSA levels of value <10 ng/mL and 60.1% for ≥10 ng/mL.* ***c****, pT stage; the PSA RFS at 5 years was 78.6% for ≤pT2 and 56.3% for ≥pT3.* ***d****, GS of the RP specimen; the PSA RFS at 5 years was 74.5% for Gleason ≤7 and 53.9% for Gleason ≥8.* ***e****, the percentage of positive biopsy cores; the PSA RFS at 5 years was 75.8% for <60% and 48.0% for ≥60%. Overall log-rank P < 0.001 in all plots. All log-rank P < 0.001.*



the present study was that we limited the patients to those whose PSA level after RP decreased to undetectable levels with no neoadjuvant/adjuvant therapy. Using this 'pure' characterized database of 1192 patients treated with RP at 37 specialized institutes in Japan, we can thus also compare the racial differences among the clinical and pathological variables and PSA recurrence with those of Caucasian and American-Africans. Pound *et al.* [5] reported a PSA increase in 16% of men (315/1997) treated by RP with no neoadjuvant/adjuvant therapy at a median follow-up of 5.3 years. Others reported that ≈35% of all men had a PSA increase within 10 years of RP [6–9]. In the present study, there was a PSA increase in 25.3% of 1192 men, thus suggesting PSA recurrence after RP to be closely similar in Japan, or even a little higher, considering the shorter follow-up. Indeed, the PSA recurrence rate within 2 years was 6.8% in the study of Pound *et al.* [5], while it was 18.4% in the present study, indicating an earlier PSA increase after RP in Japan. The reason for this was not clear, but as a potential cause, the frequency of PSA assay after RP could affect it. In Japan, serum PSA is assayed every 1–3 months after RP for several years, while it is assessed every 6–12 months in the USA [6–9]. The time to PSA recurrence after surgery should be shorter if the serum PSA value is assayed more frequently. The PSA level before RP, GS, T category and margin status reportedly provide more clinically relevant stratification of the PSA outcome with T1–T2 disease [10–12]. In the present study, significant clinicopathological factors were similarly assessed by the log-rank test and the biopsy GS, PSA level before RP, pathological stage and the GS of the RP specimen were all strongly associated with PSA recurrence after RP. There were only 21 men with pN1 disease (1.8%), which resulted in an insignificant statistical result. For the PSA level before RP, Partin *et al.* [13] reported that 64%, 50%, 35% and 16% of patients with a serum PSA level of <4, 4–10, 10–20 and >20 ng/mL, respectively, had pathologically organ-confined disease. As a result, patients with a serum PSA level of 10–20 ng/mL are at intermediate risk of PSA recurrence, while those with a serum PSA level of >20 ng/mL represent a high-risk population for developing PSA recurrence after RP [13]. Regarding the importance of the pathological stage and surgical margin status, Khan *et al.* [14] constructed a nomogram that was simple to use and divided the probability of long-term PSA recurrence-free survival (RFS) into four groups according to the RP GS, pathological stage, and surgical margin status, i.e. excellent, good, moderate and low. The PSA RFS at 10 years was 95%, 72%, 41% and 13%, respectively [14]. These data suggest that the factors associated with PSA recurrence after RP were similar in Japanese patients, and the character and bioactivity closely matched that in Western countries. In addition, there was extraprostatic extension (i.e. ≥pT3) in 391 patients (32.8%), suggesting that the understaging of clinical stage in Japan was similar to that in the USA. In the present study, 52.5% of all the cases were clinical stage T1c (Table 1). At present, a greater percentage of men might have T1c disease, but in the present study patients had RP between 1993 and 2002. In the USA, the proportion of T1c was 48.3% of the 2417 cases in 1988–2002 [15] and 63% of the 5079 cases in 1994–2000 [16]. Therefore, there were no significant differences in the staging of the patients between those in Japan and in the USA, which probably did not affect the study outcome.

For the percentage of positive biopsy cores, several previous studies developed tables for

predicting the PSA recurrence risk using biopsy tumour volume measurements [17–19]. D'Amico *et al.* [17] developed a nomogram to predict PSA recurrence using the total percentage of cores that were positive, whereas Nelson *et al.* [18] used the greatest percentage of a biopsy core involved by cancer. Freedland *et al.* [19] reported that the percentage of cores positive from the dominant side of the prostate was a better predictor of PSA recurrence than the total percentage of positive cores. The present multivariate analysis identified that the percentage of positive biopsy cores was the most significant independent factor for PSA recurrence (hazard ratio of 2.05 at a threshold of 60%; Table 3). Although the difference was significant even when the threshold was set at 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%, it was most significant when the patients were divided at 60% (data not shown). A prostate biopsy is one of the essential methods to diagnose prostate cancer, and this is often and most easily quantified by determining the percentage of the biopsy cores with cancer. The percentage of the cores, with the PSA level, pT and GS, provided significant risk stratification for PSA failure after RP.

However, the median GS was 6 in biopsy specimens but 7 in the RP specimen (Table 1), indicating that the GS tends to be lower by one grade at biopsy. A multivariate analysis with Cox's proportional regression indicated the GS of the RP specimen, but not of the biopsy, was significantly associated with PSA recurrence, which clearly indicates that the GS of the RP specimen is a reliable biomarker of PSA recurrence.

ACKNOWLEDGEMENTS

This work was supported by Health Sciences Research Grants for Clinical Research for Evidenced Based Medicine and Grants-in-Aid for Cancer Research (14S-4), from the Ministry of Health, Labor and Welfare, Japan and Grant-in-aid for scientific research (No.16591600) from Ministry of Education, Culture, Sports, Science & Technology, Japan.

CONFLICT OF INTEREST

None declared.

REFERENCES

1 **Jemal A, Tiwari RC, Murray T *et al.*** Cancer statistics, 2004. *CA Cancer J Clin* 2004; **54**: 8–29
2 **Naito S.** Evaluation and management of prostate-specific antigen recurrence after radical prostatectomy for localized prostate cancer. *Jpn J Clin Oncol* 2005; **35**: 365–74
3 **Yokomizo A, Kawamoto H, Nihei K *et al.*** Randomized controlled trial to evaluate radiotherapy +/– endocrine therapy versus endocrine therapy alone for PSA failure after radical prostatectomy: Japan Clinical Oncology Group Study JCOG 0401. *Jpn J Clin Oncol* 2005; **35**: 34–6
4 **Pettaway CA.** Racial differences in the anorogen/androgen receptor pathway in prostate cancer. *J Natl Med Assoc* 1999; **91**: 653–60
5 **Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC.** Natural history of progression after PSA elevation following radical prostatectomy. *JAMA* 1999; **281**: 1591–7
6 **Zincke H, Bergstralh EJ, Blute ML *et al.*** Radical prostatectomy for clinically localized prostate cancer. long-term results of 1,143 patients from a single institution. *J Clin Oncol* 1994; **12**: 2254–63
7 **Trapasso JG, deKernion JB, Smith RB, Dorey F.** The incidence and significance of detectable levels of serum prostate specific antigen after radical prostatectomy. *J Urol* 1994; **152**: 1821–5
8 **Catalona WJ, Smith DS.** 5-year tumor recurrence rates after anatomical radical retropubic prostatectomy for prostate cancer. *J Urol* 1994; **152**: 1837–42
9 **Pound CR, Partin AW, Epstein JI, Walsh PC.** Prostate-specific antigen after anatomic radical retropubic prostatectomy. Patterns of recurrence and cancer control. *Urol Clin North Am* 1997; **24**: 395–406
10 **Kupelian PA, Buchsbaum JC, Elshaikh M, Reddy CA, Zippe C, Klein EA.** Factors affecting recurrence rates after prostatectomy or radiotherapy in localized prostate carcinoma patients with biopsy Gleason score 8 or above. *Cancer* 2002; **95**: 2302–7
11 **Blute ML, Bergstralh EJ, Iocca A, Scherer B, Zincke H.** Use of Gleason score, prostate specific antigen, seminal vesicle and margin status to predict biochemical failure after radical prostatectomy. *J Urol* 2001; **165**: 119–25
12 **D'Amico AV, Whittington R, Malkowicz SB *et al.*** Predicting prostate specific antigen outcome preoperatively in the prostate specific antigen era. *J Urol* 2001; **166**: 2185–8
13 **Partin AW, Kattan MW, Subong EN *et al.*** Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. *JAMA* 1997; **277**: 1445–51
14 **Khan MA, Partin AW, Mangold LA, Epstein JI, Walsh PC.** Probability of biochemical recurrence by analysis of pathologic stage, Gleason score, and margin status for localized prostate cancer. *Urology* 2003; **62**: 866–71
15 **McAleer SJ, Schultz D, Whittington R *et al.*** PSA outcome following radical prostatectomy for patients with localized prostate cancer stratified by prostatectomy findings and the preoperative PSA level. *Urol Oncol* 2005; **23**: 311–7
16 **Partin AW, Mangold LA, Lamm DM, Walsh PC, Epstein JI, Pearson JD.** Contemporary update of prostate cancer staging nomograms (Partin Tables) for the new millennium. *Urology* 2001; **58**: 843–8
17 **D'Amico AV, Whittington R, Malkowicz SB *et al.*** Clinical utility of the percentage of positive prostate biopsies in defining biochemical outcome after radical prostatectomy for patients with clinically localized prostate cancer. *J Clin Oncol* 2000; **18**: 1164–72
18 **Nelson CP, Rubin MA, Strawderman M, Montie JE, Sanda MG.** Preoperative parameters for predicting early prostate cancer recurrence after radical prostatectomy. *Urology* 2002; **59**: 740–6
19 **Freedland SJ, Aronson WJ, Terris MK *et al.*** The percentage of prostate needle biopsy cores with carcinoma from the more involved side of the biopsy as a predictor of prostate specific antigen recurrence after radical prostatectomy: results from the Shared Equal Access Regional Cancer Hospital (SEARCH) database. *Cancer* 2003; **98**: 2344–50

Correspondence: Seiji Naito, Department of Urology, Graduate School of Medical Science, Kyushu University, Fukuoka 812–8582, Japan. e-mail: naito@uro.med.kyushu-u.ac.jp

Abbreviations: **GS**, Gleason score; **RP**, radical prostatectomy; **RFS**, recurrence-free survival.

APPENDIX

Participating Institutions (Urologic Oncology Study Group in the Japan Clinical Oncology Group): Hokkaido University, Sapporo Medical University, Tohoku University, Miyagi Cancer Center, Akita University, Tsukuba University, Tochigi Cancer Center, Gunma University, Chiba Cancer Center, Chiba University, National Defense Medical College, National Cancer Center Hospital, Tokyo Women's Medical School, Keio University, The Jikei University, Nippon Medical School, Kitasato University, Niigata Cancer Center Hospital, Niigata university, Yamanashi University, Shinshu University, Hamamatsu Medical School, Shizuoka Cancer Center, Nagoya University, Mie University, Kyoto University, Osaka Medical Center for Cancer and Cardiovascular Diseases, Kobe University, Nara Medical University, Shimane University, Kurashiki Central Hospital, Okayama University, Kagawa Medical University, National Shikoku Cancer Center, Kyushu University, Kurume University and Kagoshima University.

The Prostate 66:536–545 (2006)

Murine Androgen-Independent Neuroendocrine Carcinoma Promotes Metastasis of Human Prostate Cancer Cell Line LNCaP

Kohsuke Uchida,[1] Naoya Masumori,[1]* Atsushi Takahashi,[1] Naoki Itoh,[1] Kazunori Kato,[2] Robert J. Matusik,[3] and Taiji Tsukamoto[1]

[1]*Department of Urologic Surgery and Andrology, Sapporo Medical University School of Medicine, Sapporo, Japan*
[2]*Department of Molecular Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan*
[3]*Department of Urologic Surgery, Vanderbilt Prostate Cancer Center, Vanderbilt University Medical Center, Nashville, Tennessee*

BACKGROUND. Although neuroendocrine (NE) cells in prostate cancer have been speculated to accelerate the growth and progression of surrounding cancer cells, the evidence is as yet inconclusive. We investigated the effect of an NE allograft (NE-10) and its cell line, NE-CS, which were established from the prostate of the LPB-Tag 12T-10 transgenic mouse, on human prostate cancer cell line LNCaP.

METHODS. The proliferation and pulmonary metastasis of LNCaP xenografts in athymic mice with and without NE-10 allografts were evaluated. Boyden chamber assay and microarray analysis were performed to investigate changes in invasion/migration and mRNA of LNCaP cells under the influence of the NE cells, respectively.

RESULTS. NE-10 did not influence the proliferation of LNCaP. The pulmonary metastasis of LNCaP with NE-10 significantly increased compared to mice without it. The NE-CS cells accelerated the in vitro invasion/migration of adenocarcinoma cells. Increased expression of mRNA of gelsolin was observed in LNCaP cells incubated with the supernatant of NE-CS cells.

CONCLUSIONS. The NE-10 allograft promotes pulmonary metastasis of subcutaneously inoculated LNCaP cells by facilitating cell invasion. Secretions from NE cells upregulate the expression of gelsolin, which is an actin-binding protein, resulting in acceleration of the migration of LNCaP cells. *Prostate 66: 536–545, 2006.* © 2005 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; neuroendocrine; gelsolin**

INTRODUCTION

To elucidate the molecular factors that lead to loss of androgen-dependence as well as the progression of prostate cancer has become one of the major tasks of current research on prostate cancer. Recently, it has focused on epigenetic events and cellular interactions between cancer cells and the surrounding cells. The role of neuroendocrine (NE) differentiated cells in prostate cancer in particular has attracted a great deal of attention. NE cells are identified as a component of conventional prostatic adenocarcinoma, occurring in 30–100% of tumors [1,2]. Androgen ablation induces an increased number of NE cells in prostate cancer [3] and the frequency and density of NE cells are more pronounced in hormone-refractory prostate cancer [4]. Since NE cells frequently lack androgen receptors (AR), they do not respond to androgen ablation therapy. However, NE cells do secrete growth-modulating

Grant sponsor: Stiftelsen Japanese-Swedish Cooperative Foundation; Grant sponsor: Japan Society for the Promotion of Science; Grant numbers: 13671660, 16591606; Grant sponsor: NIH; Grant number: CA-76142; Grant sponsor: Frances Williams Preston Laboratory of the T.J. Martell Foundation.

*Correspondence to: Naoya Masumori, MD, Department of Urology, Sapporo Medical University School of Medicine, S-1, W-16, Chuo-Ku, Sapporo 060-8543, Japan. E-mail: masumori@sapmed.ac.jp

Received 13 May 2005; Accepted 24 August 2005
DOI 10.1002/pros.20369
Published online 21 December 2005 in Wiley InterScience (www.interscience.wiley.com).

neuropeptides. These observations have led to the hypothesis that NE cells are providers of paracrine factors that accelerate the growth and progression of surrounding prostate cancer cells toward an androgen-independent state. However, the relationship between NE cells and prostatic adenocarcinoma cells remains obscure. It is controversial whether the appearance of NE cells indicates a poor prognosis due to their ability to promote the progression of adenocarcinoma to androgen independence and metastasis [4,5].

We recently developed a transgenic mouse model of prostate cancer composed of NE carcinoma, using a recombinant gene expressing an SV40 large T-antigen (Tag) transforming sequence under the regulatory control of the rat large probasin promoter (LPB) [6]. An NE-10 allograft that is transplantable into athymic mice was established from a ventral lobe of the murine prostate [7]. This allograft exhibits NE features histologically and immunohistologically. Cells in the NE-10 allograft are weakly positive or negative for AR and the tumor shows androgen-independent growth in vivo. In addition, the in vitro cell line NE-CS was established from the NE-10 allograft [8]. The cells have characteristics of NE differentiation similar to the NE-10 allograft except they no longer express Tag. Transplantation of NE-CS cells into athymic mice indicates their tumorigenic ability.

We have reported that the NE-10 allograft promotes LNCaP (human prostate adenocarcinoma cell line) tumor progression to androgen independence [9]. LNCaP tumor growth decreases in mice bearing LNCaP alone after castration. In contrast, LNCaP tumors continue to grow in those bearing both LNCaP and NE-10 tumors. On the other hand, it remains unknown whether NE-10 is involved in the progression of prostatic adenocarcinoma in normal androgen status. In this study, we investigated whether the NE-10 allograft and NE-CS cell line affected the proliferation, adhesion, migration, invasion, and metastatic potential of LNCaP.

MATERIALS AND METHODS

Cell Cultures

The human prostate adenocarcinoma cell line LNCaP was obtained from the American Type Culture Collection, and used with passage numbers between 44 and 49. The murine prostate neuroendocrine cancer allograft (NE-10) and its cell line (NE-CS) were established from the LPB-Tag transgenic mouse line 12T-10 [7,8]. The LNCaP and NE-CS cells were maintained in culture medium [RPMI-1640 (Gibco BRL, Breda, The Netherlands) supplemented with MEM non-essential amino acid (10 ml/L, Gibco BRL), MEM sodium pyruvate, penicillin-streptomycin (10 ml/L, Gibco BRL), and containing 10% fetal bovine serum (FBS, ICN Biomedicals, Costa Mesa, CA), and 7.5% $NaHCO_3$] in 5% CO_2 in a humidified incubator.

In Vivo Study

LNCaP cells (5×10^6 suspended in 75 µl of serum-free RPMI-1640 medium) were mixed with 75 µl of Matrigel (Becton Dickinson, Sunnyvale, CA) in a syringe. These cells were subcutaneously injected into the backs of 6-week-old athymic male mice (Balb/c, nu/nu, Sankyo Labo, Tokyo, Japan). At 7 weeks after injection, the mice with LNCaP xenografts were randomly divided into two groups (LNCaP-only and LNCaP+NE-10). In the LNCaP+NE-10 group, a 50 mg tissue block of the NE-10 allograft was inoculated into the contralateral back of each mouse with LNCaP. The tumor volume of the LNCaP xenograft was measured every other week. The tumor volume (mm^3) of LNCaP was calculated by the formula 0.523 × long diameter $(mm)^2$ × short diameter (mm). At 17 weeks after injection of LNCaP cells, the mice were killed. Blood samples were taken from the ventricle, and the lungs were removed. Bromodeoxyuridine (BrdU, Roche Molecular Biochemicals, Mannheim, Germany), 1 ml/100 g (body weight), was administered into the abdominal cavity of each mouse at 1 hr before sacrifice.

The lung tissues were fixed in 10% formalin and embedded in paraffin to assess incorporation of BrdU and immunohistochemistry for prostate-specific antigen (PSA) and Tag. Microscopic images of the PSA immunostained sections were obtained using a digital camera equipped with a microscope. The images of each specimen with the largest area were processed as the whole area of the lung for analysis using NIH Image (National Institutes of Health, Bethesda, Maryland).

Serum levels of PSA were measured in blood from the mice with the NE-10 allograft and control mice by using the radioimmunoassay Tandem-R PSA (SRL, Tokyo, Japan).

Immunohistochemistry for PSA, T-Antigen, BrdU, and CD31.

Tissue sections were stained with an antibody against Tag using a technique reported previously [6]. PSA and CD31 immunostaining was performed using a rabbit polyclonal anti-human antibody against PSA (Code No. A0562, 1:2000, DakoCytomation, Glostrup, Denmark) and monoclonal anti-human antibody against CD31 (Code No. M0823, 1:40, DakoCytomation), respectively. BrdU immunostaining was performed using a 5-Bromo-2'-deoxy-uridine Labeling and Detection Kit II (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Preparation of the Medium for In Vitro Studies

The supernatant of NE-CS cells that were incubated in culture medium in 5% CO_2 in a humidified incubator for 36 hr was used as NE medium. Culture medium without NE-CS cells that was left in culture dish under the same conditions for 36 hr was prepared as control medium. NE medium and control medium were used for cell proliferation assay, cell migration/invasion assay, cell adhesion assay, microarray analysis, Northern blot analysis, and florescence staining for actin filaments.

Cell Proliferation Assay

LNCaP cells (2×10^4 cells/well) were suspended in wells of a 96-well plate. At 3 days after suspension in culture medium, it was changed to NE medium (n = 9) or control medium (n = 9). After culture for an additional 3 days, MTT assay was performed for estimating cellular viability using a commercially available kit (Cell Counting Kit-8, Dojindo Laboratories, Kumamoto, Japan).

Cell Migration and Invasion Assay

In vitro invasiveness of LNCaP was determined according to the method described by Albini et al. [10]. Briefly, in a Transwell culture chamber (Coster Science, Cambridge, MA), a polyvinylpyrrolidone-free polycarbonate filter with an 8.0 μm pore size was precoated with 5 μg of fibronectin (Biomedical Technologies, Stoughton, MA) on the lower surface and 10 μg of the reconstituted basement membrane material Matrigel (Becton Dickinson, Sunnyvale, CA) on the upper surface. The cells that invaded across the pores were counted under a microscope after hematoxylin and eosin staining. The experiments were carried out in triplicate. Cell migration assay was performed using the filter without Matrigel coating.

Two different experiments were performed. In Experiment 1, LNCaP cells (1×10^5) were harvested and placed in the upper chamber of a Boyden chamber with 100 μl of NE medium or control medium. In the lower chamber, 600 μl of culture medium was added. In Experiment 2, 600 μl of culture medium with or without NE-CS cells was incubated in the lower chamber for 36 hr and then LNCaP cells (1×10^5) suspended in 100 μl of culture medium were placed in the upper chamber. The numbers of migrating and invading cells were counted at 2, 4, 6, and 8 hr and 4, 8, 12, and 20 hr, respectively.

Cell Adhesion Assay

Harvested LNCaP cells (1×10^3) were resuspended in NE medium or control medium and plated in 24-well plates precoated with collagen type IV (Becton Dickinson, Franklin Lakes, NJ) and incubated for 1 or 2 hr. Non-adherent cells were removed by aspiration, and washed three times with PBS. The total protein content of adherent cells was determined by the BCA method (Pierce, Rockford, IL). The absorbance was determined at a wavelength of 562 nm in a model microplate reader in triplicate experiments.

Isolation of RNA and Microarray

LNCaP cells cultured in culture medium were harvested when they reached subconfluence. The LNCaP cells suspended in the NE medium or control medium were incubated. Before (0 hr) and after treatment with NE medium or control medium for 4 hr and 8 hr, total RNA was extracted using an RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA microarray analysis was conducted by Kurabo Corp. Target preparation, CodeLink™ DNA microarray hybridization (Amersham Bioscience, Piscataway, NJ), and processing were performed as described previously [11]. The fragmented target cRNA was used for hybridization of each UniSet Human 1 Expression Bioarray chip. In this analysis, 1.5-fold up or downregulation was defined as a significant change.

Northern Blot Analysis

To confirm the reliability of results obtained from the microarray, Northern blot analysis was performed using total RNA derived from LNCaP cells at 4 hr and 8 hr after incubation with NE medium or control medium. Electrophoresis, blotting, and hybridization were done according to a standard protocol. Complementary DNA probes were generated by RT-PCR from total RNA of LNCaP cells. After amplification by the conventional PCR technique (*Gelsolin*: forward 5′-ACTGGTCTACTGTGTCTCTA-3′ and reverse 5′-TCTTCAGCCCACACTTTCTG-3′), the PCR products were purified using a Gel Extraction Kit (Qiagen) and a random hexamer labeled with alkaline phosphatase (Amersham AlkphosDirect). The hybridization signals were detected with CDP-Star Chemiluminescent Detection Reagent and exposed to hyperfilm (Amersham). Ethidium bromide staining of 28 S RNA was used to compare the amounts of loading.

Fluorescence Staining for Actin Filaments

Harvested LNCaP cells were resuspended in NE medium or control medium and incubated in a 2-well culture slide precoated with fibronectin for 5 hr (Becton Dickinson, Franklin Lakes, NJ). After discarding the medium, adherent cells were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 for