

Use of Irinotecan for Treatment of Small Cell Carcinoma of the Prostate

Wei-Lin Tung,^{1,2,3} Yuwei Wang,^{1,2} Peter W. Gout,² Dean-Mo Liu,³
Martin Gleave,¹ and Yuzhuo Wang^{1,2*}

¹The Vancouver Prostate Centre, Vancouver General Hospital and Department of Urologic Sciences, the University of British Columbia, Vancouver, British Columbia, Canada

²Department of Experimental Therapeutics, BC Cancer Agency, Vancouver, British Columbia, Canada

³Department of Materials Science and Engineering, National Chiao Tung University, Hsinchu, Taiwan, ROC

BACKGROUND. Prostatic small cell carcinoma (SCC) is a rare variant of prostate cancer. It is extremely aggressive and resistant to available therapies with a median survival range of 5–17 months. No standard chemotherapeutic regimen has been established for its treatment. In search of a new therapeutic approach, we examined the response of patient-derived prostatic SCC *tissue* xenografts to irinotecan, a topoisomerase I inhibitor.

METHODS. A tumor tissue line was established from a patient's prostatic SCC by subrenal capsule grafting using NOD-SCID mice. Mice carrying subcutaneous transplants of the tumor line were then treated for 2 weeks with irinotecan alone and in combination with cisplatin. The effect on tumor volume, histopathology, and apoptosis were determined.

RESULTS. The prostatic SCC tissue line resembled the donor tissue in morphologic and immunohistochemical features. Irinotecan (20 mg/kg/day; days 1–3, 8–10) completely arrested xenograft growth with a small reduction in tumor volume and only minor weight loss of the hosts (7%); irinotecan (12 mg/kg; same schedule) + cisplatin (2.5 mg/kg/day; days 1 and 8) had a similar effect, but with lower weight loss. While the growth inhibition involved apoptosis, it was also associated with a marked increase in autophagy.

CONCLUSIONS. Tumor tissue lines established via subrenal capsule xenografting provide models with clinical relevance and the present study suggests that irinotecan could be useful for therapy of refractory prostatic SCC, in particular in combination with cisplatin. *Prostate* 71: 675–681, 2011. © 2010 Wiley-Liss, Inc.

KEY WORDS: small cell carcinoma; prostate; irinotecan; subrenal capsule xenografts; autophagy

INTRODUCTION

Small cell carcinoma (SCC) of the prostate is a rare form of prostate cancer comprising 0.5–2% of all prostate carcinomas [1]; it was first described by Wenk et al. [2]. Prostatic SCC can occur as an initial event at the time of first diagnosis and is then thought to originate from neuroendocrine cells present in the epithelium of prostatic ducts/acini and in the endothelium of prostatic urethra. It may also develop in prostate cancer during progression to castration resistance induced by androgen ablation. The most widely accepted view is that prostatic SCCs arise from totipotential stem cells of the prostate that have the

ability to differentiate into either epithelial or neuroendocrine type carcinomas. The latter type is characterized by absence of expression of androgen receptors (AR) and prostate-specific antigen (PSA) and by

Grant sponsor: Canadian Institutes of Health Research.

*Correspondence to: Yuzhuo Wang, PhD, Department of Experimental Therapeutics, BC Cancer Agency—Research Centre, 675 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3.

E-mail: ywang@bccrc.ca

Received 29 July 2010; Accepted 31 August 2010

DOI 10.1002/pros.21283

Published online 14 October 2010 in Wiley Online Library (wileyonlinelibrary.com).

positive expression of the synaptophysin (SYN) neuroendocrine marker [3,4]. Prostatic SCC is an aggressive disease, often described as universally fatal [5]. It is typically diagnosed at an advanced clinical stage with rapid progression and insensitivity to hormonal therapy; patients have a median survival of 5–17 months [6,7]. Prostatic SCC shows similarity to small cell lung carcinoma (SCLC) in morphologic features [6] and in the expression pattern of some genes [8]. In view of this, the commonly used treatment of prostatic SCC, that is, platinum-based chemotherapy and irradiation, has been adopted from SCLC therapeutic modalities. Various anti-cancer agents have been used in combination with cisplatin, including etoposide, cyclophosphamide, doxorubicin, and vincristine; other combinations used are carboplatin plus etoposide or taxanes [9–12]. Cisplatin-based chemotherapy plus radiotherapy has, despite initial positive responses, failed to be effective and a standard therapeutic regimen for prostatic SCC has not yet been adopted since this disease is extremely rare [3]. At present, chemotherapy using cisplatin in combination with etoposide is the most common treatment for prostatic SCC.

In the field of preclinical cancer therapy there is a critical need for models which are relevant to the human disease. Use of mouse xenograft models to represent clinical cancers has become widespread, as they are reasonably inexpensive, able to provide rapid data, largely free of regulatory constraints and can be used with many different tumor types. Although *cell line* xenograft models, generated by injecting cultured human cancer cells, are frequently used, they have profound disadvantages [13]. Typically, the cancer cells used are derived from advanced, highly aggressive or poorly differentiated neoplasms [14] and the developing tumors lack their original microenvironment, including tumor-associated stroma shown to be important in tumor development and progression [15]. Such cancer cell line xenograft models in general show limited ability for predicting clinical efficacy of anticancer agents [16]. To address these limitations, mice can be used carrying xenografts of patient-derived *tumor tissue*. Such models are more representative of the clinical situation since they tend to maintain the anatomical architecture and function of the original tumors. While tumor tissue xenografts are generally based on subcutaneous (s.c.) implantation of cancer tissue, we have adopted subrenal capsule grafting of cancer tissues into NOD-SCID mice to allow optimal establishment of cancer tissue lines. The subrenal capsule graft site allows very high tumor engraftment rates of various human cancers, including prostatic carcinoma. It permits high tissue perfusion and hence promotes viability and growth of the xenografts with

retention of tumor heterogeneity, that is, retention of major histopathologic and genetic characteristics of the original cancers, making them useful for drug response evaluation [17–20]. Once established, tumor tissue lines can be used to develop s.c. tumors whose volumes can be readily measured with calipers.

Irinotecan is a topoisomerase I inhibitor which prevents DNA from unwinding. It is a prodrug which is converted to a biologically active metabolite, 7-ethyl-10-hydroxy-camptothecin (SN-38), by a carboxylesterase-converting enzyme [21]. It is mainly used for treatment of colorectal cancer, but also for advanced SCLC. Importantly, in a randomized phase III trial of the latter, cisplatin plus irinotecan showed a better survival rate for patients with good performance status than cisplatin plus etoposide [22,23]. In the present study, we have focused on irinotecan as a potential drug for treating prostatic SCC using xenografts of a tumor tissue line obtained via subrenal capsule grafting of human prostatic SCC in NOD-SCID mice. Evidence was obtained that irinotecan, particularly in combination with cisplatin, is potentially useful for treatment of prostatic SCC.

MATERIALS AND METHODS

Materials and Animals

Chemicals, solvents and solutions were obtained from Sigma–Aldrich (Oakville, ON, Canada) unless otherwise indicated. Six- to 8-week-old NOD-SCID mice were bred in the BC Cancer Research Centre Animal Resource Centre, BC Cancer Agency, Vancouver, Canada. Animal care and experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Development of a Prostatic SCC Tissue Line for Drug Evaluation

A patient with prostatic adenocarcinoma [Gleason score of 9 (5 + 4)] underwent radical cystoprostatectomy and androgen ablation therapy at the Vancouver General Hospital (Vancouver, Canada). Three months later, because of deteriorating obstructive voiding symptoms, he underwent additional surgery to remove enlarged urethra in which prostatic SCC was identified. A piece of the SCC specimen was obtained with his informed consent following a protocol approved by the University of British Columbia Clinical Research Ethics Board/BC Cancer Agency. A portion of the fresh tumor tissue was frozen at -80°C , some of the tissue was fixed in 10% neutral buffered formalin and embedded in paraffin; the majority of the tissue was used to develop a tumor tissue line using subrenal capsule grafting technology and serial transplantations, as previously

described [18,24]. Following establishment of the tumor line (after three serial transplantations), pieces of xenograft tissue ($3 \times 3 \times 1$ mm) were grafted subcutaneously into mice (four grafts/mouse). After 5 weeks (to allow establishments of the xenografts) the mice were randomly distributed into groups with at least five mice per group and treated for 2 weeks with irinotecan (Sandoz Inc, Princeton, NJ; 20 mg/kg/day), days 1–3, 8–10, and with a combination of irinotecan and cisplatin (Mayne Pharma, Paramus, NJ): irinotecan (12 mg/kg/day), days 1–3, 8–10 and cisplatin (2.5 mg/kg/day), days 1 and 8, administered as i.p. injections; control animals were injected with similar volumes of saline. Drug dosages and treatment schedules were obtained from published studies [25]. Tumor sizes were measured with calipers (mm) on days 1 and 14; all xenografts were harvested on day 14.

Histopathology and Immunohistochemistry

The donor specimens and xenograft tissues were fixed in 10% neutral buffered formalin, processed through graded alcohols and embedded in paraffin. Sections were cut at a thickness of 4 μ m, mounted on glass slides and dewaxed in Histoclear (National Diagnostic, Atlanta, GA), and hydrated in graded alcohol solutions and distilled water. For histopathology, routine H&E staining was carried out. For immunohistochemistry, tissue sections were treated with 0.5% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidase activity, followed by washing in PBS (pH 7.4) and incubation with a blocking solution (ImmunoVision Technology, Springdale, AR) for 30 min. The sections were then incubated with the primary antibodies overnight at 4°C or with non-immune mouse IgG (Zymed Corp., San Francisco, CA), used as negative controls, at the same concentration as the primary antibodies. Primary antibodies used: mouse anti-AR (AR; Santa Cruz Biotechnology, Santa Cruz, CA; 1:50), mouse anti-PSA (human PSA; DAKO, Carpinteria, CA; 1:50), rabbit anti-SYN (SYN; Abcam Inc, Cambridge, MA), mouse anti-Ki-67 (DAKO; 1:50), rabbit anti-caspase-3 (Cell Signaling, Danvers, MA; 1:100), and rabbit anti-LC3B (microtubule-associated protein light chain 3, LC3B; Abcam; 1:500). Following incubation, the tissue sections were washed, incubated with either a biotinylated anti-rabbit or anti-mouse antibody (Amersham International, Arlington Heights, IL), and then incubated with avidin–biotin complex (Vector Laboratories, Burlingame, CA) at room temperature. Immunoreactivity was visualized using 3,3-diaminobenzidine in PBS and 0.03% H₂O₂. All sections used for immunohistochemistry were lightly counterstained with 5% (w/v) Harris hematoxylin and dehydrated in graded alcohols.

Analysis and Statistics

For quantification of Ki-67 and caspase-3 immunostaining of cells, five randomly selected high-power ($\times 400$) images from each graft were captured using an AxioCam HRCCD mounted on an Axioplan 2 microscope, using Axiovision 3.1 software (Carl Zeiss). The percentages of Ki-67- or caspase-3-positive cells (index) were calculated using the formula: Index = number of positive cells \times 100/number of total cells. Viable tumor areas of the treated and control groups were averaged and presented as means \pm SD. *P*-values were calculated using a permutation test of the means. Ki-67 and caspase-3 indices are presented as means \pm SD and analyzed by the Student's *t*-test. Results with *P*-values < 0.05 are considered statistically significant.

RESULTS

Establishment of a Transplantable Prostatic SCC Tissue Line

Subrenal capsule xenografting of the patient's prostatic SCC tissue in NOD-SCID mice (as described in Materials and Methods Section) was successful with an overall engraftment rate $> 95\%$. Within 8 months, a transplantable tumor tissue line was established that was non-metastatic when grafted into the subrenal capsule site. The established tumor line (designated LTL-352) grew readily following transplantation into the s.c. graft site in NOD-SCID mice with an approximate doubling time of 11 days. LTL-352 tissue of the established line and of earlier generations was stored frozen in liquid nitrogen to allow checking/use of the tumor tissue at a later date. As shown in Figure 1, comparisons of donor and xenograft tissues showed no major differences with regard to characteristic morphologic and immunohistochemical features, such as growing patterns (Fig. 1a,b), absence of AR expression (Fig. 1c,d), absence of PSA expression (Fig. 1e,f) and positive expression of SYN neuroendocrine marker (Fig. 1g,h). Both donor and xenograft cells showed patterns of cells in sheets and nests, with ribbons, pallasading along fibrous bands; rosette-like structures were occasionally noted. Polygonal, round, or spindle tumor cells had scanty cytoplasm with hyperchromatic nuclei. Taken together, the results show that the human prostatic SCC xenografts in general retained the histopathology and differentiation status of the donor tissues. The data indicate that during the early generations of transplantation (e.g., the fourth generation) the human prostatic SCC line did not show morphological changes in the mouse host and thus was phenotypically quite stable. Furthermore, mice carrying xenografts of the tissue line could be used as models with clinical relevance.

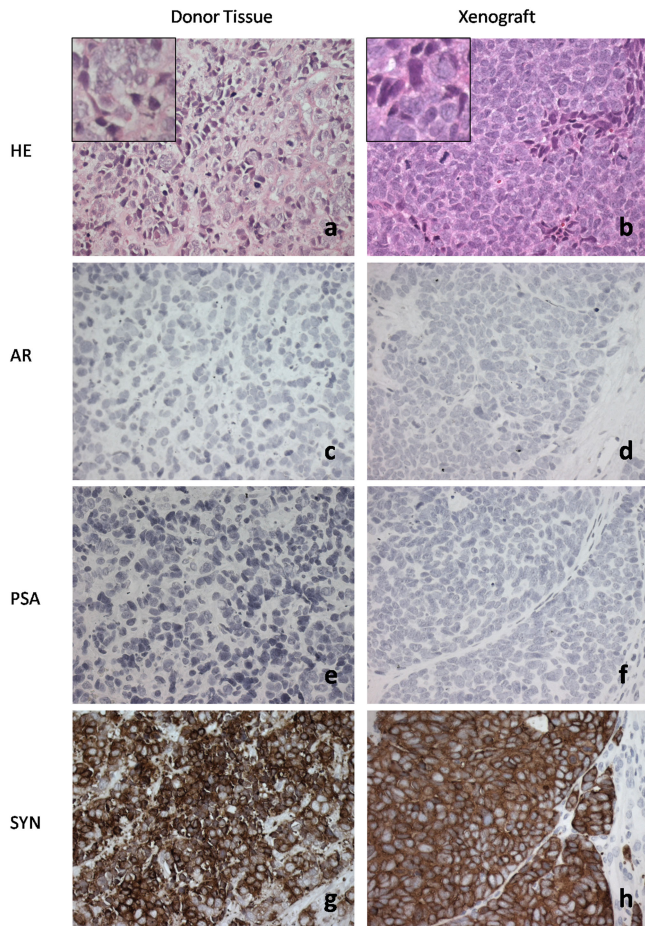


Fig. 1. Histopathology of representative prostatic SCC donor and xenograft tissues. Tissues were stained with H&E (HE; **a,b**), and using antibodies against androgen receptor (AR; **c,d**), prostate-specific antigen (PSA; **e,f**), or synaptophysin (SYN; **g,h**). Insets, high-power images ($\times 400$).

Response of Prostatic SCC Xenografts to Chemotherapeutic Regimens

The anti-cancer activities of irinotecan, and a combination of irinotecan plus cisplatin, were determined using mice bearing s.c. LTL-352 prostatic SCC xenografts. The drugs were administered intraperitoneally after the average volume of the tumors had reached 100–200 mm³. As shown in Figure 2a, the growth of the tumors was markedly inhibited both by irinotecan as a single agent ($P < 0.01$) and by irinotecan + cisplatin ($P < 0.01$). In the control group, the average tumor volume increased over the 2-week period from 154.7 ± 22.3 mm³ to 349.8 ± 50.4 mm³ (126.1%); in contrast, the tumor volume was reduced in the irinotecan-treated group from 130.9 ± 12.8 mm³ to 106.3 ± 9.9 mm³ (–19%), and from 180.7 ± 22.9 mm³ to 153.4 ± 25.4 mm³ (–15%) when cisplatin was used in addition to irinotecan (Fig. 2a). The treated/control

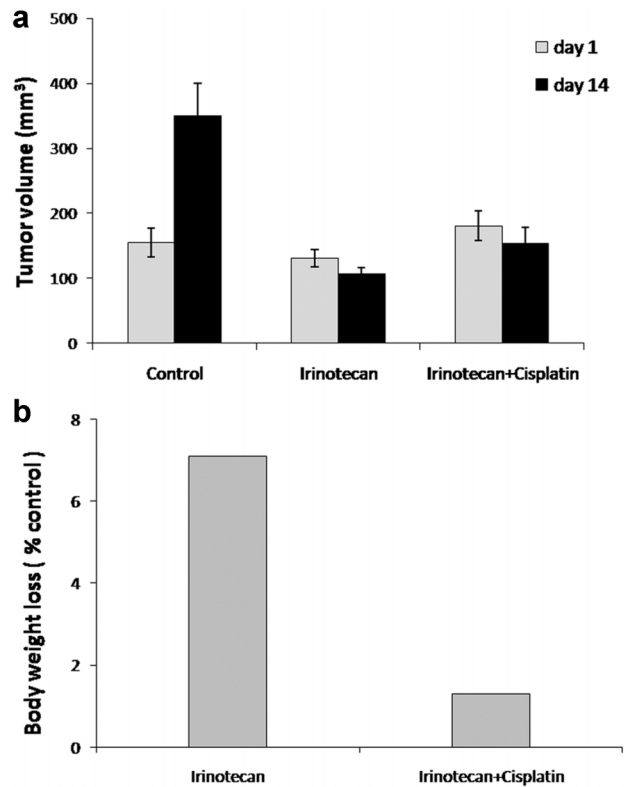


Fig. 2. Effect of treatment with irinotecan and irinotecan + cisplatin on prostatic SCC LTL-352 subcutaneous xenografts in NOD-SCID mice. Injections (i.p.) were given of saline (control), irinotecan (20 mg/kg/day; days 1–3, 8–10) or a combination of irinotecan (12 mg/kg/day; days 1–3, 8–10) and cisplatin (2.5 mg/kg/day; days 1 and 8) over a 14-day period. **a:** Effect on tumor volumes on days 1 and 14; data are expressed as mean \pm SD. **b:** Body weight loss on day 14 as compared to controls (%).

value (T/C) was –12.6% for the mice treated with irinotecan alone (dosage, 20 mg/kg/day) and –14.0% for the mice treated with irinotecan + cisplatin (at 12 and 2.5 mg/kg/day, respectively). The regimens did not cause any deaths nor significant toxic side-effects. Compared to control animals, weight loss was 7.1% for the mice treated with irinotecan alone and 1.3% for the mice treated with irinotecan + cisplatin (Fig. 2b, $P > 0.05$). The results indicate that the treatment with irinotecan + cisplatin was more effective and less toxic than the treatment with irinotecan as a single agent.

Histopathological analysis was used to determine the effect of the drugs at the cellular level. A comparison with the control tissues shows that the tissues of both irinotecan- and irinotecan + cisplatin-treated tumors contained more apoptotic bodies, in particular the irinotecan + cisplatin-treated tumors (Fig. 3a–c). A marked increase was found for the staining of caspase-3 (a marker of apoptosis) for both treated groups (Fig. 3d–f), especially in the irinotecan + cisplatin-treated tumors (Fig. 3f, $P < 0.01$), as also

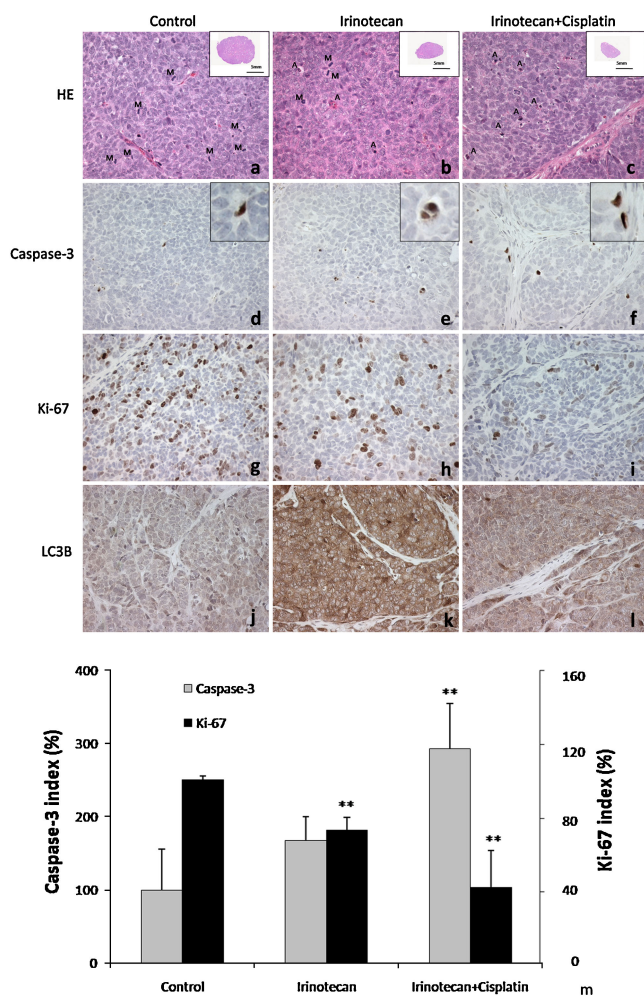


Fig. 3. Immunohistochemical analysis of prostatic SCC LTL-352 tissues from control (untreated), irinotecan-treated and irinotecan + cisplatin-treated xenografts. **a–c:** Apoptotic bodies (M, mitotic activity; A, apoptosis); **d–f:** caspase-3 expression; **g–i:** Ki-67 expression; **j–l:** LC3B expression; insets, $\times 400$ magnification; **m,** caspase-3 and Ki-67 indices compared to controls for irinotecan- and irinotecan + cisplatin-treated xenografts. Asterisks indicate that $P < 0.01$.

indicated in Figure 3m. Ki-67 activity (a marker of cell proliferation) was reduced especially in the irinotecan + cisplatin-treated tumors (Fig. 3i, $12.1 \pm 5.6\%$, $P < 0.01$) compared to the mice treated with irinotecan alone (Fig. 3h, $19.4 \pm 1.9\%$, $P < 0.01$, the proliferative index for the control group was $26.7 \pm 2.8\%$; Fig. 3g,m). Both treated groups showed an increase in expression of LC3B (a marker of autophagy) compared to the control (Fig. 3j), in particular in the tumors treated with irinotecan alone (Fig. 3k). The results indicate that the anti-cancer activity of irinotecan and cisplatin involved apoptosis and that irinotecan may have a role in autophagy.

DISCUSSION

Prostatic SCC is a rare type of prostate cancer. However, it is of clinical importance since it is one of the most aggressive prostatic malignancies with very poor prognosis. Cisplatin-based chemotherapy plus radiotherapy, the most commonly applied regimen in the clinic, is only marginally effective, despite initial positive tumor responses, and new therapeutic approaches are critically required [3]. In the present preclinical study, we focused on use of irinotecan as this drug is a component of a first-line treatment of extensive SCLC [23], a disease that has a number of features in common with prostatic SCC [6,8]. Mice carrying xenografts of the patient-derived LTL-352 prostatic SCC tumor tissue line, showing retention of histopathological and immunohistochemical features of the original cancer (Fig. 1), provided a model with clinical relevance. The finding that irinotecan completely arrested the growth of the xenografts, with a small reduction in tumor volume (Fig. 2) and minor toxicity as indicated by a body weight loss of only $\sim 7\%$, indicates that this drug is potentially useful for clinical therapy of prostatic SCC. In fact, a combination of cisplatin with irinotecan at a lower dosage may be even more useful, since such a combination led to similar growth arrest of the xenografts with negligible body weight loss (Fig. 2). A greater effectiveness of irinotecan + cisplatin, compared to irinotecan as a single agent, is also indicated by the lower T/C value (-14% vs. -12%), lower Ki-67 values, and increased caspase-3 expression (Fig. 3m). It may be noted that, in a case study, combined chemotherapy using carboplatin plus irinotecan has shown favorable efficacy in a patient with relapsed prostatic SCC complicated with meningeal carcinomatosis [26].

It is of interest that irinotecan has been reported to reduce cellular levels of glutathione by interference with cellular cystine uptake [27]. Reductions in cellular glutathione levels can lead to sensitization of cells to anticancer agents [28,29]. In view of this, irinotecan could, as a reducer of intracellular glutathione levels, be a valuable component in combination chemotherapy of refractory cancers such as prostatic SCC.

Growth-inhibitory activity of irinotecan in cancer cell lines is thought to be mediated by apoptosis induced by SN-38, the active form of irinotecan [30]. The increase in apoptotic bodies and caspase-3 staining observed in cells of the tumors whose growth was arrested by irinotecan (Fig. 3a–f) indicates that the growth inhibition, as shown by tumor volume (Fig. 2) and Ki-67 labeling measurements (Fig. 3m), was also due to induction of apoptosis. Of further interest is the increase in autophagy of the treated tumors as indicated by an increase in LC3B marker expression, in

particular in the tumors treated with irinotecan alone (Fig. 3j–l). The increase in autophagy appears to be due to irinotecan as distinct from cisplatin, as the reduced LC3B expression could be explained by the 40% reduction of irinotecan dosage in the irinotecan + cisplatin regimen. The role of autophagy in cancer is complex, since there is evidence that autophagy supports both promotion and suppression of cancer growth. There is an increasing amount of evidence that autophagy can act as a survival mechanism of cells in response to a wide range of stresses, including treatment with anti-cancer agents [31,32]. To our knowledge, this is the first report that activity of irinotecan may be coupled to autophagy.

CONCLUSIONS

A transplantable tumor tissue line has been established from a patient's prostatic SCC, via subrenal capsule xenografting in NOD-SCID mice, which provides a clinically relevant model for this disease. Using the model it was found that irinotecan can be useful for therapy of prostatic SCC, in particular in combination with cisplatin.

ACKNOWLEDGMENTS

This study was supported by a grant from the Canadian Institutes of Health Research (Y.Z.W./M.G.). The authors thank Rebecca Wu, Hui Xue for excellent technical assistance and Xin Dong for reviewing the histopathology slides.

REFERENCES

- Lopez-Barcons LA. Small-cell neuroendocrine carcinoma of the prostate: Are heterotransplants a better experimental model? *Asian J Androl* 2010;12(3):308–314.
- Wenk RE, Bhagavan BS, Levy R, Miller D, Weisburger W. Ectopic ACTH, prostatic oat cell carcinoma, and marked hypernatremia. *Cancer* 1977;40(2):773–778.
- Stein ME, Bernstein Z, Abacioglu U, Sengoz M, Miller RC, Meirovitz A, Zouhair A, Freixa SV, Poortmans PH, Ash R, Kuten A. Small cell (neuroendocrine) carcinoma of the prostate: Etiology, diagnosis, prognosis, and therapeutic implications—A retrospective study of 30 patients from the rare cancer network. *Am J Med Sci* 2008;336(6):478–488.
- Hansson J, Abrahamsson PA. Neuroendocrine pathogenesis in adenocarcinoma of the prostate. *Ann Oncol* 2001;12 (Suppl 2): S145–S152.
- Moore SR, Reinberg Y, Zhang G. Small cell carcinoma of prostate: Effectiveness of hormonal versus chemotherapy. *Urology* 1992;39(5):411–416.
- Oesterling JE, Hauzeur CG, Farrow GM. Small cell anaplastic carcinoma of the prostate: A clinical, pathological and immunohistological study of 27 patients. *J Urol* 1992;147(3 Pt 2):804–807.
- Tetu B, Ro JY, Ayala AG, Johnson DE, Logothetis CJ, Ordonez NG. Small cell carcinoma of the prostate. Part I. A clinicopathologic study of 20 cases. *Cancer* 1987;59(10):1803–1809.
- Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, Macvicar GR, Varambally S, Harwood J, Bismar TA, Kim R, Rubin MA, Pienta KJ. Androgen-independent prostate cancer is a heterogeneous group of diseases: Lessons from a rapid autopsy program. *Cancer Res* 2004;64(24):9209–9216.
- Aoki H, Ishidoya S, Ito A, Endoh M, Shimazui T, Arai Y. Experience of the treatment with gemcitabine, docetaxel, and carboplatin (GDC) chemotherapy for patients with small-cell carcinoma of the prostate. *Int J Urol* 2006;13(9):1254–1258.
- Palmgren JS, Karavadia SS, Wakefield MR. Unusual and underappreciated: Small cell carcinoma of the prostate. *Semin Oncol* 2007;34(1):22–29.
- Papandreou CN, Daliani DD, Thall PF, Tu SM, Wang X, Reyes A, Troncoso P, Logothetis CJ. Results of a phase II study with doxorubicin, etoposide, and cisplatin in patients with fully characterized small-cell carcinoma of the prostate. *J Clin Oncol* 2002;20(14):3072–3080.
- Blunt DM, Sansom HE, King DM. Imaging of small cell carcinoma of the male urogenital tract. *Clin Radiol* 1996;51(10): 724–727.
- Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. *Nat Protoc* 2007;2(2):247–250.
- Phelps RM, Johnson BE, Ihde DC, Gazdar AF, Carbone DP, McClintock PR, Linnoila RI, Matthews MJ, Bunn PA, Jr., Carney D, Minna JD, Mulshine JL. NCI-Navy Medical Oncology Branch cell line data base. *J Cell Biochem Suppl* 1996;24:32–91.
- Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature* 2004;432(7015):332–337.
- Voskoglou-Nomikos T, Pater JL, Seymour L. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. *Clin Cancer Res* 2003;9(11): 4227–4239.
- Wang Y, Revelo MP, Sudilovsky D, Cao M, Chen WG, Goetz L, Xue H, Sadar M, Shappell SB, Cunha GR, Hayward SW. Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. *Prostate* 2005; 64(2):149–159.
- Lee CH, Xue H, Sutcliffe M, Gout PW, Huntsman DG, Miller DM, Gilks CB, Wang YZ. Establishment of subrenal capsule xenografts of primary human ovarian tumors in SCID mice: potential models. *Gynecol Oncol* 2005;96(1):48–55.
- Cutz JC, Guan J, Bayani J, Yoshimoto M, Xue H, Sutcliffe M, English J, Flint J, LeRiche J, Yee J, Squire JA, Gout PW, Lam S, Wang YZ. Establishment in severe combined immunodeficiency mice of subrenal capsule xenografts and transplantable tumor lines from a variety of primary human lung cancers: Potential models for studying tumor progression-related changes. *Clin Cancer Res* 2006;12(13):4043–4054.
- Griffin TW, Bogden AE, Reich SD, Antonelli D, Hunter RE, Ward A, Yu DT, Greene HL, Costanza ME. Initial clinical trials of the subrenal capsule assay as a predictor of tumor response to chemotherapy. *Cancer* 1983;52(12):2185–2192.
- Rivory LP, Bowles MR, Robert J, Pond SM. Conversion of irinotecan (CPT-11) to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by human liver carboxylesterase. *Biochem Pharmacol* 1996;52(7):1103–1111.
- Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S, Saijo N. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 2002;346(2):85–91.

23. Jiang J, Liang X, Zhou X, Huang L, Huang R, Chu Z, Zhan Q. A meta-analysis of randomized controlled trials comparing irinotecan/platinum with etoposide/platinum in patients with previously untreated extensive-stage small cell lung cancer. *J Thorac Oncol* 2010;5(6):867–873.
24. Wang Y, Xue H, Cutz JC, Bayani J, Mawji NR, Chen WG, Goetz LJ, Hayward SW, Sadar MD, Gilks CB, Gout PW, Squire JA, Cunha GR, Wang YZ. An orthotopic metastatic prostate cancer model in SCID mice via grafting of a transplantable human prostate tumor line. *Lab Invest* 2005;85(11):1392–1404.
25. Nagano T, Yasunaga M, Goto K, Kenmotsu H, Koga Y, Kuroda J, Nishimura Y, Sugino T, Nishiwaki Y, Matsumura Y. Antitumor activity of NK012 combined with cisplatin against small cell lung cancer and intestinal mucosal changes in tumor-bearing mouse after treatment. *Clin Cancer Res* 2009;15(13):4348–4355.
26. Yamada T, Ohtsubo K, Mouri H, Yamashita K, Yasumoto K, Izumi K, Zen Y, Watanabe H, Yano S. Combined chemotherapy with carboplatin plus irinotecan showed favorable efficacy in a patient with relapsed small cell carcinoma of the prostate complicated with meningeal carcinomatosis. *Int J Clin Oncol* 2009;14(5):468–472.
27. Chintala S, Toth K, Yin MB, Bhattacharya A, Smith SB, Ola MS, Cao S, Durrani FA, Zinia TR, Dean R, Slocum HK, Rustum YM. Downregulation of cystine transporter x(c) by irinotecan in human head and neck cancer FaDu xenografts. *Chemotherapy* 2010;56(3):223–233.
28. Lo M, Wang YZ, Gout PW. The x(c)-cystine/glutamate antiporter: A potential target for therapy of cancer and other diseases. *J Cell Physiol* 2008;215(3):593–602.
29. Lo M, Ling V, Low C, Wang YZ, Gout PW. Potential use of the anti-inflammatory drug, sulfasalazine, for targeted therapy of pancreatic cancer. *Curr Oncol* 2010;17(3):9–16.
30. Oizumi S, Isobe H, Ogura S, Ishida T, Yamazaki K, Nishimura M, Kawakami Y, Dosaka-Akita H. Topoisomerase inhibitor-induced apoptosis accompanied by down-regulation of Bcl-2 in human lung cancer cells. *Anticancer Res* 2002;22(6C):4029–4037.
31. Chen N, Debnath J. Autophagy and tumorigenesis. *FEBS Lett* 2010;584(7):1427–1435.
32. Corcelle EA, Puustinen P, Jaattela M. Apoptosis and autophagy: Targeting autophagy signalling in cancer cells—'Trick or treats'? *FEBS J* 2009;276(21):6084–6096.