Cervical Cancer Patient-Derived Orthotopic Xenograft (PDOX) Is Sensitive to Cisplatinum and Resistant to Nab-paclitaxel

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Abstract. *Background: Cervical cancer is a world-wide problem that requires transformative therapeutic strategies. We have previously developed patient-derived orthotopic xenograft (PDOX) nude-mouse models of this disease. In the present report, we demonstrate that the standard drug, cisplatinum (CDDP), is highly-effective while the new, highly-touted agent, nab-paclitaxel (NAB-PTX) is ineffective. Materials and Methods: Cervical PDOX tumors were grown on the cervix of nude mice for 4 weeks after surgical orthotopic implantation (SOI). Tumors were treated with CDDP or NAB-PTX. Results: H&E staining demonstrated that the PDOX tumor recapitulated the original patient tumor. CDDP was highly-effective. One tumor that was treated with CDDP completely regressed. CDDP-treated tumors were smaller (tumor volume ratio: 0.42±0.36) than* the control group (tumor volume ratio: 3.47±1.66) (p<0.01). In contrast, NAB-PTX did not show significant efficacy on the cervical cancer PDOX model (tumor volume ratio: 2.85±1.45) (p=0.47). CDDP-treated tumor weight (50±50 mg) was significantly less than control (238±114 mg) (p<0.01). NAB-PTX-treated tumors were not reduced in weight (246±136 mg) compared to control (p=0.91). There were no significant differences in mouse body weight between groups. Histological evaluation demonstrated that CDDP-treated tumors were fibrotic with scattered squamous cell nests compared to control or NAB-PTX-treated tumors. Conclusion: The results of the present study demonstrate the power of PDOX models of cervical cancer to distinguish efficacy of potential therapeutics for individual patients with this disease.*

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Key Words: Cervical cancer, patient-derived othotopic xenograft, PDOX, nude mice, drug response, cispatinum, nab-paclitaxel.

Cervical cancer is the second-most common cancer worldwide in women. The majority is classified as squamous-cell carcinoma (SCC). SCC resulted in 454,000 cases and 200,000 deaths in 2010 (1). Chemotherapy drugs used for cervical cancer include nanoparticle albumin-bound (nab)-paclitaxel (NAB-PTX), carboplatinum, cisplatinum (CDDP), bleomycin, mitomycin-C, vincristine, and irinotecan (2). Retinoids and interferon in combination with cytotoxic chemotherapy have been shown to be effective for SCC (3). However, there is no standard treatment for metastatic cervical cancer.

Clinically-relevant mouse models of cancer can be used for tailor-made therapy based on the patient-derived tumor.

Our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI), including pancreatic (4-7), breast (8), ovarian (9), lung (10), cervical (11), colon (12-14), stomach (15), sarcoma (16-20) and melanoma (21).

We have previously developed mouse models of patient cervical cancer. Tumors in nude mice had histological structures similar to the original tumor and were stained by anti-HER2 antibody in the same pattern as the patient's cancer. In the PDOX model of HER2-positive cervical cancer, carboplatinum was active. However, the most active regimen was the combination of tumor-targeting *Salmonella typhimurium* A1-R and trastuzumab (22).

Recently, patient-derived xenograft cervical cancer mouse models were also described where the aggressiveness of the patient tumor was replicated in mouse models (23). NAB-PTX is paclitaxel linked to albumin nanoparticles, which makes it soluble. The development of nanotechnology as a delivery system for NAB-PTX has improved the pharmacokinetics and pharmacodynamics of paclitaxel, in part by decreasing its hydrophobicity (24). In the present study, we compared the efficacy of two drugs for cervical cancer, CDDP and NAB-PTX in a cervical-cancer PDOX model.

Materials and Methods

Animals. Female athymic (*nu/nu*) nude mice (AntiCancer, Inc., San Diego, CA, USA), 4-6 weeks old, were used in this study. Mice were kept in a barrier facility under HEPA filtration. Mice were fed with an autoclaved laboratory rodent diet. All mouse surgical procedures and imaging were performed with the animals anesthetized by intramuscular injection of a 0.02 ml solution of 50% ketamine, 38% xylazine, and 12% acepromazine maleate. All animal studies were conducted with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study and in accordance with the principals and procedures outlined in the National Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873–1 (11).

Specimen collection. The patient provided written informed consent and the tumor specimen was procured under the approval of the Institutional Review Board of Kawasaki Medical School.

Patient characteristics. The patient was a 57-year-old female with primary cervical cancer. Histology demonstrated squamous cell carcinoma (grade 2) (Figure 1C). The patient received no previous treatment. A radical hysterectomy was performed with bilateral salpingo-oophorectomy and pelvic lymphadenectomy.

Establishment of a cervical cancer PDOX model with surgical orthotopic implantation (SOI). A fresh resected primary tumor was obtained and transported immediately to the laboratory on ice. The specimen was cut into 5 mm fragments, which were implanted subcutaneously to nude mice. Three months later, implanted tumors grew to more than 10 mm in diameter. The established tumors were cut into 4 mm^3 fragments. After anethesizing mice, a 7 mm lower abdominal midline incision was made, and then the uterine cervix was exposed. A single fragment was implanted in the cervix of each mouse using 8-0 nylon sutures (Ethilon, Ethicon, Inc., NJ, USA). The wound was closed with 6-0 nylon sutures (Ethilon, Ethicon, Inc., NJ, USA).

Treatment study design in the PDOX model of cervical cancer. Treatment protocol. G1: control group treated with vehicle (*i.v.*, phosphate buffered saline (PBS), once a week, 3 weeks, n=7); G2: test group treated with CDDP (*i.v.*, 5 mg/kg, once a week, 3 weeks, n=7); G3: test group treated with NAB-PTX (*i.v.*, 10 mg/kg, twice a week, 3 weeks, n=7). Treatment started four weeks after orthotopic implantation. Mice were sacrificed on day 22, then tumors were resected for further evaluation. Tumor length and width were measured both on day 0 and day 22. Tumor volume was calculated with the following formula: Tumor volume (mm^3) = length (mm) × width (mm) × width (mm) × 1/2. Tumor volume ratio was defined as the ratio of volume on day 22 to day 0.

Tumor tissue was removed with surrounding normal tissues at the time of resection. The tissues were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (3 μm) were deparaffinized in xylene and rehydrated in an ethanol series. PDOX tumors were also evaluated with frozen section. Tumors were divided into 2-3 mm fragments, then embeded in Neg-50 Frozen Section Medium (Thermo Fisher Scientific, Waltham, MA). The embedded tissues were immediately frozen with liquid nitrogen. Tissue sections (8 μm) were made with a cryostat. Hematoxylin and eosin (H&E) staining was performed according to standard protocols and examined using an BH-2 microscope (Olympus Corporation, Japan) equipped with a INFINITY1 2.0 megapixel CMOS digital camera (Lumenera Corporation, Canada). All images were acquired using INFINITY ANALYZE software (Lumenera Corporation) without post-acquisition processing (11).

Statistical analysis. SPSS statistics version 21.0 was used for all statistical analyses (IBM, New York, NY, USA). Significant differences for continuous variables were determined using the Student's *t*-test. Data are presented as average ± SD. Bar graphs expressed average, and error bars show SD. A probability value of $P<0.05$ was considered statistically significant.

Results

Cervical PDOX tumors were grown on the cervix of nude mice for 4 weeks after SOI (Figure 1A). H&E demonstrated that the PDOX tumors recapitulated the original patient tumor (Figure 1B, C). CDDP was highly-effective on the cervical cancer PDOX. One tumor treated with CDDP completely regressed. CDDP-treated tumors were significantly smaller (tumor-volume ratio: 0.42±0.36) than control-group tumor (tumor volume-ratio 3.47±1.66) ($p<0.01$) (Figure 2A). In contrast, NAB-PTX did not have efficacy on the cervical-cancer PDOX model (tumor volume ratio: 2.8±1.45) ($p=0.47$). CDDP-treated tumor weight (50±50 mg) was significantly less than control (238±114 mg) ($p<0.01$) (Figure 2B). NAB-PTX-treated tumors were not reduced in weight (246±136 mg) compared to control ($p=0.91$). There were no significant differences in mouse body weight among groups (Figure 2C). Histological
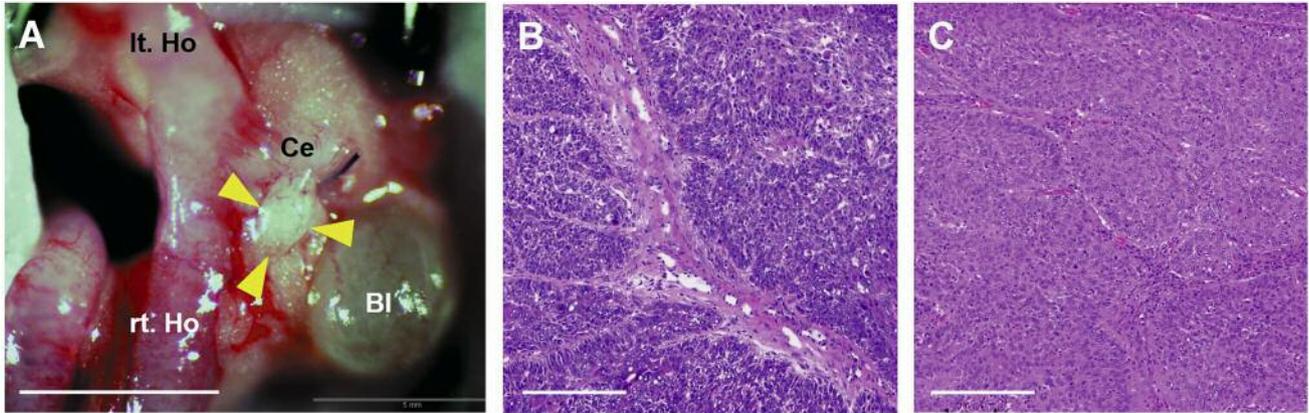
Figure 1. *Cervical-cancer patient-derived orthotopic xenograft (PDOX) (A) PDOX tumor was grown on the cervix four weeks after surgical orthotopic implantation (SOI). Arrowheads indicate the PDOX cervical tumor; rt. Ho, right uterine horn; lt. Ho, left uterine horn; Ce, uterine cervix; Bl, urinary bladder; scale bar, 5 mm (B) H&E staining of a PDOX tumor in the control group; scale bar, 200 μm (C) H&E staining of patient's original tumor; scale bar, 200 μm.*
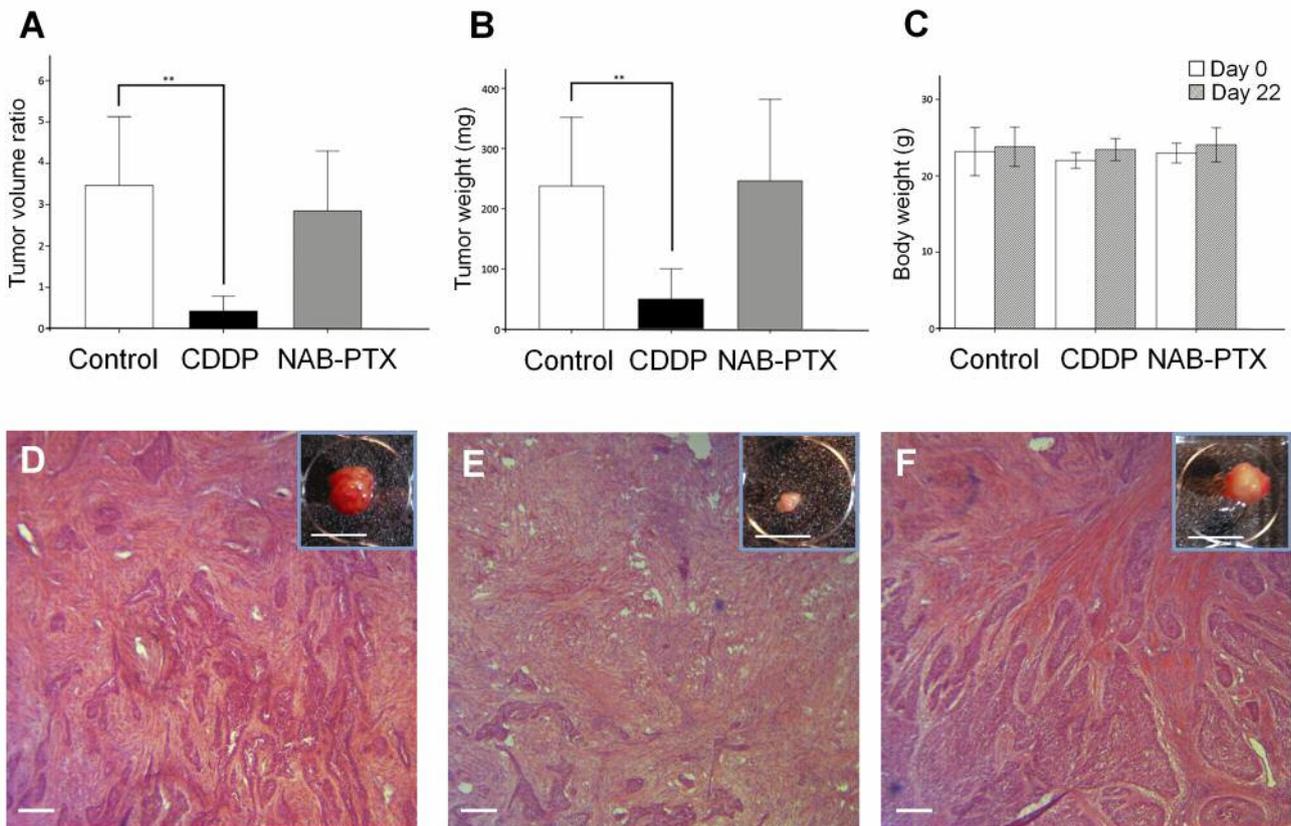

Figure 2. *Treatment efficacy of chemotherapeutic drugs in a cervical cancer PDOX model (A) Bar graph shows tumor volume ratio that was defined as the ratio of volume on day 22 to day 0. (B) Bar graph shows tumor weight on day 22. (C) Mouse body weight did not differ, both at the start and end of the study. Resected specimens and their H&E-stained histology in control (D), CDDP (E) and NAB-PTX (F) groups. Two asterisks indicate p-value <0.01; error bars: ±1 SD; scale bars, 10 mm for specimens inserts, 200 μm for histology (panels D-F); CDDP, cisplatinum; NAB-PTX, nab-paclitaxel.*

evaluation demonstrated that CDDP-treated tumors were fibrotic with scattered squamous-cell nests compared to control or NAB-PTX-treated tumors (Figures 2D-F).

The result of the present study is of particular importance, as it demonstrates the high efficacy on the PDOX model of a low-price generic drug, CDDP, and lack of efficacy of a high-cost patented drug, NAB-PTX.

Discussion

Our present and previous results (11, 22) together with the results of Rofstad *et al.* (23) indicate the potential important clinical use of patient-derived models in order to individualize and optimize treatment of this, often recalcitrant, disease. The results in the present show that an improvement on best-guess treatment could be achieved with the use of patient-derived models.

Dedication

This paper is dedicated to the memory of A.R. Moossa, M.D. and Sun Lee, M.D.

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