Estrogen receptor-β signaling induces cisplatin resistance in bladder cancer

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Abstract: The efficacy of cisplatin-based chemotherapy in patients with bladder cancer is often limited due to the development of therapeutic resistance. Our recent findings in bladder cancer suggested that activation of prostaglandin receptors (e.g. EP2, EP4) or cyclooxygenase (COX)-2 induced cisplatin resistance. Meanwhile, emerging evidence indicates the involvement of estrogen receptor-β (ERβ) signals in urothelial cancer progression. In this study, we aimed to investigate whether ERβ activity was associated with cisplatin sensitivity in bladder cancer. Immunohistochemistry in muscle-invasive bladder cancer specimens from 55 patients who had subsequently received at least 3 cycles of cisplatin + gemcitabine neoadjuvant chemotherapy showed that ERβ was positive in 38% of responders vs. 71% of non-responders (P = 0.016), including 42% of male responders vs. 65% of male non-responders (P = 0.142) and 20% of female responders vs. 100% of female non-responders (P = 0.048). Then, cisplatin cytotoxicity was compared in human bladder cancer cell lines. Control sublines endogenously expressing ERβ were significantly more resistant to cisplatin treatment at its pharmacological concentrations, compared with ERβ knockdown sublines via short hairpin RNA virus infection. An ER modulator tamoxifen increased sensitivity to cisplatin in ERα-negative/ERβ-positive cell lines, while, in an estrogen-depleted condition, 17β-estradiol reduced it. Additionally, western blot showed considerable elevation in ERβ expression in cisplatin-resistant bladder cancer sublines, compared with respective controls. Moreover, treatment with tamoxifen or a COX-2 inhibitor celecoxib increased cisplatin sensitivity even in resistant cells, while COX-2/EP2/EP4 inhibitor treatment resulted in reduced expression of ERβ. The expression and activity of β-catenin known to involve cisplatin resistance was also up-regulated in cisplatin-resistant cells, which was further induced by 17β-estradiol treatment. The present results suggest that estrogen-mediated ERβ signaling plays an important role in modulating cisplatin sensitivity in bladder cancer cells. Targeting ERβ during chemotherapy may thus be a useful strategy to overcome cisplatin resistance especially in female patients with ERβ-positive bladder cancer.

Keywords: Bladder cancer, cisplatin, chemoresistance, estrogen receptor, immunohistochemistry

Introduction

Bladder cancer is one of the most commonly diagnosed malignancies worldwide [1]. Clinically, there are two distinct forms: non-muscle-invasive and muscle-invasive diseases. Muscle-invasive bladder cancer is often associated with metastatic disease which still has a poor prognosis with a relative 5-year overall survival rate of 5.5% [2]. While several immune checkpoint agents have recently been approved for clinical use, cisplatin (CDDP)-based combination chemotherapy remains the first-line treatment for advanced bladder cancer [3, 4]. However, the response rate to CDDP-containing regimen has been reported to be 40-60% in patients who underwent neoadjuvant chemotherapy followed by radical cystectomy [5, 6]. In addition, the underlying mechanisms for CDDP resistance in bladder cancer remain poorly
understood. Therefore, the development of strategies for not only overcoming chemoresistance but also predicting chemosensitivity is urgently required. Indeed, we have recently demonstrated preclinical findings in bladder cancer suggesting that activation of prostaglandin receptors (e.g. EP2, EP4) and cyclooxygenase (COX)-2 [7], as well as androgen receptor (AR) [8], is associated with induction of CDDP resistance.

Various epidemiological and clinical studies have indicated that bladder cancer incidence is three- to four-fold higher in men than in women, while women tend to be diagnosed with more advanced stage tumors and have higher mortality from bladder cancer [9]. To understand these sex-related differences, steroid hormone receptors, such as AR and estrogen receptor (ER), have been investigated if they contribute to urothelial carcinogenesis and cancer progression [10, 11]. The ER has two major isoforms, ERα and ERβ. Of the two, ERβ has been shown to be physiologically expressed in a variety of organs, including the bladder [12]. It has also been documented that ERβ is expressed, as a favorable prognosticator, in neoplastic conditions, such as cancers of the breast [13], colon [14], ovary [15], prostate [16], and stomach [17], while its prognostic significance remains controversial in some other malignancies, such as esophageal cancer, non-small cell lung cancer, and endometrial cancer [18-21]. ERβ expression has also been associated with worse prognosis in patients with pancreatic cancer [22] as well as bladder cancer [23]. Specifically, in bladder cancer, a meta-analysis showed that ERβ expression was significantly up-regulated in high-grade or muscle-invasive tumors, compared with low-grade or non-muscle-invasive tumors, respectively, and was significantly associated with a higher risk of recurrence of non-muscle-invasive tumors (hazard ratio = 1.573; 95% confidence interval = 1.102-2.247; P = 0.013) [24]. Meanwhile, ERα is undetectable in most of urothelial tumors [23, 24], and its role in bladder cancer may thus be limited.

In preclinical models for bladder cancer, ERβ has been shown to promote tumor progression [25-28]. In particular, 17β-estradiol (E2) promotes the proliferation of ERα-negative and ERβ-positive bladder cancer cells via, for instance, inducing the expression of BCL2 and cyclin D1 genes [29] or inactivating a potential tumor suppressor FOXO1 [30]. However, the impact of ERβ signaling on modulating CDDP sensitivity is not fully understood. In the present study, we aimed to investigate whether ERβ activation could be associated with CDDP resistance in bladder cancer.

Material and methods

Antibodies and chemicals

Anti-ERβ (H-150 or B-3) and anti-GAPDH (6C5) antibodies, and an anti-β-catenin antibody were purchased from Santa Cruz Biotechnology and BD Biosciences, respectively. CDDP, E2, and tamoxifen (TAM) were obtained from Sigma-Aldrich, celecoxib and ONO-AE3-208 (EP4 antagonist) were from ChemScene, and AH6809 (EP2 antagonist) was from Cayman Chemical.

Cell lines

Human urothelial carcinoma cell lines (UMUC3, 5637) were originally obtained from the American Type Culture Collection. Another human urothelial carcinoma cell line (647V) was used in our previous studies [7, 8, 30-32]. All these lines were recently authenticated, using GenePrint 10 System (Promega). In these cell lines, ERβ-shRNA lentiviral particles (sc-35325-V; Santa Cruz Biotechnology) or control shRNA lentiviral particles (sc-108080; Santa Cruz Biotechnology) were stably expressed. Additionally, as previously described [7, 8], CDDP-resistant sublines were established by stepwise, continuous treatment with CDDP (e.g. 0.2-2.0 μM) for at least 12 weeks. UMUC3/647V- and 5637-derived cells were maintained in Dulbecco’s modified Eagle’s medium (Gibco) and RPMI 1640 (Mediatech), respectively, supplemented with 10% fetal bovine serum (FBS), penicillin (50 U/mL), and streptomycin (50 μg/mL) at 37°C in a humidified atmosphere of 5% CO2. Cells were cultured in phenol red-free medium supplemented with 10% charcoal-stripped FBS for E2 treatment at least 24 hours before experimental treatment.

Western blot

Equal amounts of proteins (30 μg) obtained from cell extracts were separated in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoro-
ride membrane electronically, blocked, and incubated with a specific antibody and a secondary antibody (anti-mouse or anti-rabbit IgG HRP-linked antibody; Cell Signaling Technology), followed by scanning with an imaging system (ChemiDoc™ MP, Bio-Rad).

**MTT assay**

Cells (5-8 × 10^3) seeded in 96-well plates were cultured for 72 h in the absence or presence (0.1-20 µM) of CDDP and then incubated with 0.5 mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich) in 100 µL of medium for 4 h at 37°C. MTT was solved by 150 µL of DMSO, and the absorbance was measured at a wavelength of 570 nm with background subtraction at 630 nm. Fifty percent inhibitory concentration (IC50) is calculated using the web-based tool (https://www.aatbio.com/tools/ic50-calculator).

**Reporter gene assay**

Cells at a density of 80% confluence in 96-well plates were co-transfected with 100 ng Topflash reporter plasmid DNA (plasmid 12456 M50 Super 8x TOPFlash containing seven TCF/LEF binding sites; Addgene) or control Fopflash reporter plasmid DNA (plasmid 12457 M51 Super 8x FOPFlash containing six mutated TCF/LEF binding sites; Addgene), along with 1 ng pRL-CMV renilla luciferase plasmid DNA, using Lipofectamine 3000 transfection reagent (Thermo Fisher Scientific). After 24 h of transfection, the cells were cultured in the presence or absence of ligands (E2/TAM) for 24 h. Cell lysates were then assayed for luciferase activity determined, using a Dual-Luciferase Reporter Assay kit (Promega) and a luminometer (Synergy 2; BioTek Instruments). The ratio of Topflash: Fopflash values normalized by renilla activity was used as an indicator of Wnt/β-catenin activity.

**Immunohistochemistry**

Upon appropriate approval from the Institutional Review Board including the request to waive the documentation of patient consent, we previously constructed a set of tissue microarray (TMA) consisting of retrieved bladder cancer tissue specimens obtained by transurethral resection [7, 8, 23, 33]. All these specimens were high-grade muscle-invasive urothelial carcinomas from patients who had subsequently received neoadjuvant chemotherapy (i.e. at least 3 cycles of CDDP + gemcitabine therapy). Responders (n = 24) and non-responders (n = 31) to the neoadjuvant therapy were pathologically defined as the absence (≤ pT1NOM0) and presence (≥ pT2, pN1-3, and/or M1), respectively, of muscle-invasive, extravesical, or metastatic disease at the time of radical cystectomy [33].

Immunohistochemistry was performed on the sections (5 µm thick) from the bladder TMA, using a primary antibody to ERβ (dilution 1:200) and a broad spectrum secondary antibody (Invitrogen), as described previously [23, 34]. All stains were manually quantified by a single pathologist (H.M.) blinded to sample identity. The immunoreactive scores calculated by multiplying the percentage of immunoreactive cells (0% = 0; 1-10% = 1; 11-50% = 2; 51-80% = 3; 81-100% = 4) by staining intensity (negative = 0; weak = 1; moderate = 2; strong = 3) were considered negative (0; score < 2), weakly positive (1+; 2 ≤ score ≤ 4), moderately positive (2+; 4 < score ≤ 8), and strongly positive (3+; score > 8).

**Statistical analysis**

Student’s t-test or one-way analysis of variance (ANOVA) was used to assess differences in variables with a continuous distribution. The Fisher exact test or chi-square test was used to evaluate the associations between categorized variables. P values less than 0.05 was considered statistically significant. All statistical analyses were performed, using EZR software (Jichi Medical University Saitama Medical Center) [35], a graphical user interface for R version 3.2.2 (The R Foundation for Statistical Computing).

**Results**

**Associations of ERβ expression in surgical specimens with chemosensitivity**

We first immunohistochemically stained for ERβ in transurethral resection specimens from bladder cancer patients who had subsequently received CDDP-based neoadjuvant chemotherapy. ERβ signals were predominantly detected in the nuclei of tumor cells (Figure 1). Overall, ERβ was positive in 31 (56%; 21 1+, 7 2+, 3 3+)
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Figure 1. Immunohistochemistry of ERβ in bladder cancer specimens. The staining is scored by a combination of the intensity [i.e. negative (A), weak (B), moderate (C), strong (D)] and distribution (i.e. percentage of immunoreactive cells). Original magnification: ×200.

Table 1. The expression of ERβ in bladder cancer and response to chemotherapy

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of 55 cases (Table 1). ERβ-positive cases consisted of 38% of responders vs. 71% of non-responders (P = 0.016). These included 42% of male responders vs. 65% of male non-responders (P = 0.142) as well as 20% of female responders vs. 100% of female non-responders (P = 0.048). ERβ immunoreactivity was thus associated with chemoresistance, especially in female patients.

Associations of ERβ expression in cell lines with CDDP sensitivity

We previously demonstrated that human bladder cancer cell lines, including UMUC3, 647V, and 5637, expressed ERβ, but not ERα [30, 36]. To assess the impact of the expression or activity of ERβ on CDDP sensitivity, we first established knockdown sublines by stably expressing ERβ-shRNA (Figure 2A). We then compared the cytotoxic effects of CDDP between ERβ-positive and ERβ-negative sublines, using MTT assay. CDDP inhibited the cell viability in a dose-dependent manner, and ERβ knockdown cells were more sensitive to CDDP treatment at its pharmacological concentrations (e.g. 1.3-8.4 μM [37]), compared with respective control sublines (Figure 2B). In these experiments, the impact of ERβ knockdown on cell growth, irrespective of CDDP, was excluded by comparing with that of mock treatment (without CDDP) in each subline. We also compared ERβ-positive lines with versus without treatment of an anti-estrogen TAM (Figure 2C) or an estrogen E2 (Figure 2D). TAM increased sensitivity to CDDP in ERα-negative/ERβ-positive cell lines, while, in an estrogen-depleted condition, E2 reduced it. Again, the effects of TAM or E2 on cell growth were excluded by comparing with versus without CDDP treatment in each line/treatment.

In our previous study, inhibition of COX-2, EP2, or EP4 was found to increase CDDP sensitivity in bladder cancer cells [7]. We further investigated the effect of a COX-2 inhibitor, an EP2 antagonist, and an EP4 antagonist on ERβ. Western blot showed that these inhibitors reduced ERβ expression in a dose-dependent manner (Figure 3).

ERβ expression in CDDP-resistant cells

To further investigate the involvement of ERβ signals in CDDP resistance in bladder cancer cells, we established CDDP-resistant sublines...
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A

B

UMUC3

647V

5637

C

D

UMUC3

647V

5637

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**Figure 2.** Effects of ERβ inactivation on CDDP cytotoxicity in bladder cancer cells. A. Western blotting of ERβ in UMUC3/647V/5637-control-shRNA vs. UMUC3/647V/5637-ERβ-shRNA cells. GAPDH served as an internal control. B. MTT assay in UMUC3/647V/5637-control-shRNA vs. UMUC3/647V/5637-ERβ-shRNA cells cultured with various concentrations (0-20 μM) of CDDP for 72 h. IC50s of CDDP are: 3.14 μM (UMUC3-control-shRNA) vs. 2.01 μM (UMUC3-ERβ-shRNA); 2.25 μM (647V-control-shRNA) vs. 1.78 μM (647V-ERβ-shRNA); and 1.80 μM (5637-control-shRNA) vs. 1.22 μM (5637-ERβ-shRNA). C. MTT assay in UMUC3/647V/5637 cells treated with ethanol (mock) vs. 1 μM TAM and various concentrations (0-20 μM) of CDDP for 72 h. IC50s of CDDP are: 4.02 μM (mock) vs. 0.91 μM (TAM) in UMUC3; 2.36 μM (mock) vs. 2.07 μM (TAM) in 647V; and 3.74 μM (mock) vs. 2.95 μM (TAM) in 5637. D. MTT assay in UMUC3/647V/5637 cells cultured in phenol red-free medium supplemented with 10% charcoal-stripped FBS containing ethanol (mock) vs. 1 μM TAM and various concentrations (0-20 μM) of CDDP for 72 h. IC50s of CDDP are: 3.63 μM (mock) vs. 4.78 μM (E2) in UMUC3; 3.60 μM (mock) vs. 9.66 μM (E2) in 647V; and 5.12 μM (mock) vs. 6.07 μM (E2) in 5637. In MTT assays, cell viability is presented relative to that of each line/treatment without CDDP, and data are expressed as mean ± SE from at least 4 replicates. *P < 0.05 (vs. control-shRNA subline or mock treatment).

**Figure 3.** Effects of COX-2/EP2/EP4 inhibitors on ERβ expression in bladder cancer cells. Western blotting of ERβ in 647V/5637 cells cultured with celecoxib (0-1 μM) (A), AH6809 (0-10 μM) (B), or ONO-AE3-208 (0-10 μM) (C) for 24 h. GAPDH served as an internal control.

by long-term culture with low/increasing doses of CDDP. The growth rates of the CDDP-resistant sublines were similar to those of control sublines cultured for the same period without CDDP (figure not shown). We then confirmed significantly lower sensitivity to CDDP in resistant sublines, compared with respective controls (Figure 4A). In these CDDP-resistant sublines, treatment with TAM or celecoxib did not significantly change the cell viability in the absence of CDDP (Figure 4B). However, TAM or celecoxib increased CDDP sensitivity, while no synergistic effects of TAM and celecoxib on CDDP cytotoxicity were seen (Figure 4C). Western blot further showed enhanced ERβ expression in CDDP-resistant cells, compared with respective controls (Figure 4D).

**Modulation of β-catenin by ERβ**

β-Catenin has been implicated in CDDP resistance in several types of malignancies, such as lung and ovarian cancers [38, 39]. We there-
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Figure 4. Effects of TAM and celecoxib on CDDP cytotoxicity in CDDP-resistant bladder cancer cells. (A) MTT assay in 647V/5637-control vs. 647V/5637-CDDP-resistant (R) cells cultured with CDDP (0-20 μM) for 72 h. IC50s of CDDP are: 2.85 μM (647V-control) vs. 8.78 μM (647V-CDDP-R); and 5.16 μM (5637-control) vs. 6.67 μM (5637-CDDP-R). (B) MTT assay in 647V/5637-CDDP-R cells cultured with ethanol (mock), TAM (1 μM), and/or celecoxib (1 μM) for 72 h. (C) MTT assay in 647V/5637-CDDP-R cells cultured with ethanol (mock), TAM (1 μM), and/or celecoxib (1 μM) in the presence of CDDP (0-20 μM) for 72 h. IC50s of CDDP are: 8.57 μM (mock) vs. 5.96 μM (TAM), 9.18 μM (mock) vs. 7.58 μM (celecoxib), and 9.18 μM (mock) vs. 7.41 μM (celecoxib + TAM) in 647V-CDDP-R; and 5.68 μM (mock) vs. 4.96 μM (TAM), 5.61 μM (mock) vs. 5.15 μM (celecoxib), and 5.61 μM (mock) vs. 3.62 μM (celecoxib + TAM) in 5637-CDDP-R. Cell viability is presented relative to that of each subline without CDDP (A, C) or with mock treatment (B). Data are expressed as mean ± SE of at least 4 replicates (A, C) or 3 independent experiments (B). *P<0.05 (vs control subline or mock treatment). (D) Western blotting of ERβ in 647V-control vs. 647V-CDDP-R and 5637-control vs. 5637-CDDP-R cells. GAPDH served as an internal control.

Therefore assessed the expression and activity of β-catenin in CDDP-resistant bladder cancer cells. Western blot showed a higher level of β-catenin expression in 5637-CDDP-resistant cells than in control cells (Figure 5A). E2 treatment resulted in an increase in β-catenin expression in CDDP-resistant cells, which was at least partially restored by TAM (Figure 5B). To further confirm whether Wnt/β-catenin signaling could be activated by ERβ, a luciferase reporter assay was performed in the extracts of cells transfected with plasmids harboring optimal TCF/LEF binding sites (Topflash) or mutated TCF/LEF binding sites (Fopflash). E2 increased Topflash/Fopflash activity and TAM reduced it in both control and CDDP-resistant sublines (Figure 5C), while the changes were not statistically significant. These results suggest that
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In addition, the canalicular multispecific organic anion transporter/MRP2 pump which effluxes CDDP, metallothionein which detoxifies heavy metal ions, glutathione which forms conjugates and detoxifies CDDP, and excision repair cross-complementing group 1 protein and the XPF protein both of which are involved in the nucleotide excision repair pathway have been implicated in CDDP resistance in bladder cancer [42]. The involvement of miRNA, lncRNA, and circRNA in CDDP resistance has also been documented [43-45]. This study provides another potential mechanism responsible for CDDP resistance by demonstrating that ERβ knockdown or ERβ blockade improved CDDP sensitivity in bladder cancer cells, while estrogen treatment reduced it. Even in CDDP-resistant cells, anti-estrogen treatment restored the sensitivity. Thus, ERβ blockade has the potential of being an option for enhancing the efficacy of CDDP therapy. Moreover, ERβ expression in CDDP-resistant sublines was found to be considerably elevated, compared with respective control sublines. ERβ positivity in surgical specimens was also associated with resistance to CDDP-based neoadjuvant chemotherapy, suggesting that ERβ immunohistochemistry in transurethral resection specimens could serve as a predictor of chemosensitivity in bladder cancer patients. This may otherwise prompt early use of immune checkpoint inhibitors besides potential combination therapy with anti-estrogen and CDDP, especially in patients with ERβ-positive tumor.

ERβ modulates the activity of Wnt/β-catenin signaling in bladder cancer cells.

Discussion

CDDP is one of the most commonly used drugs for the treatment of a variety of malignancies. However, due to acquired resistance, tumors often progress even if combination therapy with other anti-cancer agent(s) is performed. Several pathways related to CDDP resistance are known. For example, extracellular-signal regulated kinase (ERK1/2), phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) or p38 mitogen-activated protein kinases (MAPK), and a tumor suppressor p53 have been shown to involve the cellular reaction to CDDP in several types of malignancies including bladder cancer [40, 41]. CDDP efflux induced by two copper transporting p-type adenosine triphosphatases (ATP7A and ATP7B), p-glycoprotein which is a product of the MDR1, and multidrug resistance protein (MRP) have also been suggested as mechanisms for CDDP resistance in non-urothelial cancers [42].

β-Catenin is a multifunctional protein which plays an important role in regulating cadherin-mediated cell adhesion and other cellular functions, such as proliferation, apoptosis, differentiation, motility, and survival, through the Wnt
pathway [46, 47]. Activated β-catenin translocates to the nucleus to form complexes with TCF/LEF and thereby activates Wnt target genes [48]. It has been documented that β-catenin induces the development and progression of several types of malignancies, such as colon, liver, pancreatic, lung, and ovarian cancers [49]. In bladder cancer, several agents and miRNAs have been shown to inhibit or promote tumor growth through the Wnt/β-catenin pathway [50]. Meanwhile, we have previously demonstrated that androgens activate β-catenin via the AR pathway in bladder cancer cells and thereby promote their growth [51]. Overexpression of β-catenin has also been known to correlate with CDDP resistance in lung cancer [38], ovarian cancer [39], and oral [52] or head and neck [53] squamous cell carcinoma. Moreover, IncRNA and miRNA could modulate CDDP sensitivity through β-catenin signals in colorectal and prostate cancers [54, 55]. Similar to our observations in CDDP-resistant bladder cancer sublines, β-catenin was highly expressed in lung adenocarcinoma and ovarian cancer cell lines resistant to CDDP [38, 39]. Additionally, in bladder cancer cells, miR-374a inhibited β-catenin while increased CDDP sensitivity [56]. Indeed, in bladder cancer patients, activated Wnt/β-catenin signaling could predict resistance to chemoradiation with CDDP [57]. Thus, our present data showing higher expression/activity of β-catenin in CDDP-resistant cells support its crucial role in CDDP resistance in bladder cancer.

Associations between ERβ and β-catenin signals have been reported in several types of malignancies. In breast cancer, ERβ overexpression or E2 treatment inhibited Wnt/β-catenin signaling [58]. In colorectal cancer [59] and osteosarcoma [60], ERβ acted as a tumor suppressor through inhibiting Wnt/β-catenin. Additionally, in prostate cancer cells, both activation and inhibition of β-catenin by ERβ signals have been demonstrated [61-63]. Although physical interactions between ERβ and β-catenin have not been documented in bladder cancer, we found that estrogen/ERβ considerably induced the expression and activity of β-catenin in CDDP-resistant bladder cancer cells.

It is well known that persistent inflammation can induce tumorigenesis in various organs [64]. The expression of COX-2, an enzyme responsible for inflammation, has been shown to be elevated in bladder cancer [65]. We have previously demonstrated not only that a COX-2 inhibitor celecoxib, as well as antagonists of prostaglandin receptors EP2 and EP4 that are downstream mediators of COX-2 [66], could inhibit urothelial carcinogenesis and bladder cancer cell viability/migration, but also that these COX-2/EP2/EP4 inhibitors could enhance the cytotoxic effects of CDDP in bladder cancer cells [7]. Functional interplay between ERβ and COX-2 signals has also been reported. Specifically, E2 significantly induced or reduced COX-2 expression in lung epithelial cells [67] or colonic epithelial cells [68], respectively. In colon cancer cells, E2 inhibited COX-2 by inactivating the Akt and ERK1/2 signaling pathway [69]. Additionally, in prostate cancer cells, overexpression of COX-2 resulted in suppression of ERβ activity and thereby increased cell motility [70]. In the present study, a COX-2 inhibitor or an EP2 or EP4 antagonist was found to reduce ERβ expression in bladder cancer lines. Moreover, celecoxib alone (or TAM alone) induced CDDP cytotoxicity in CDDP-resistant bladder cancer cells, while no synergistic effect of TAM and celecoxib on CDDP sensitivity was observed.

In conclusion, the present study provides evidence suggesting that estrogen-mediated ERβ signaling plays an important role in modulating CDDP sensitivity in bladder cancer. Targeting ERβ via, for instance, anti-estrogen treatment during CDDP therapy may thus be a useful chemosensitization strategy especially in female patients with ERβ-positive bladder cancer. Furthermore, ERβ expression in bladder cancer specimens may precisely predict chemoresponse.

**Disclosure of conflict of interest**

None.

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