Hormone Receptors in Bladder Cancer

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Accumulating evidence indicates that sex hormone receptors, such as androgen receptor and estrogen receptors, play an important role in modulating sensitivity to conventional non-surgical therapy for bladder cancer.

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1. Introduction

Intravesical therapy with bacillus Calmette-Guérin (BCG) or chemotherapeutic agents, such as doxorubicin, mitomycin C, and thiotepa, has been widely used in the management of non-muscle-invasive bladder cancer after transurethral surgery. In patients with localized muscle-invasive bladder cancer, systemic cisplatin-based chemotherapy and/or radiotherapy have been employed before or after radical cystectomy. In addition, systemic chemotherapy or an antibody against programmed cell death-1 (PD-1) or its ligand (PD-L1) can be used in those with more advanced/metastatic disease.

Sex hormone receptors, such as androgen receptor (AR), estrogen receptors (ERs) (i.e., ERα, ERβ), and progesterone receptor, are a group of steroid receptors that are activated upon binding of cognitive ligands, androgens, estrogens, and progestogens, respectively. Recent findings indicate a vital role of sex hormone receptor signals in the pathogenesis of urothelial cancer. Specifically, AR activation has been implicated in urothelial tumorigenesis, whereas conflicting results exist as to the estrogen effects that may be dependent on the functional activity of ERα versus ERβ in urothelial cells. Meanwhile, emerging data suggest the association of androgen/estrogen-mediated receptor activity with the therapeutic efficacy in patients with bladder cancer.

2. Sex Hormone Receptor Signaling and Sensitivity to Conventional Non-Surgical Treatment for Bladder Cancer

2.1. Chemotherapy

In bladder cancer specimens from patients who had subsequently received cisplatin-based neoadjuvant chemotherapy, there was a correlation between AR immunoreactivity and chemosensitivity. In bladder cancer sublines resistant to cisplatin, as well as gemcitabine, AR expression has been found to be considerably elevated, compared to control or parental cells. These findings suggest the involvement of AR signals in chemoresistance in bladder cancer.

Cell proliferation assay data indeed showed that bladder cancer lines with AR overexpression or androgen treatment were more resistant to cisplatin. Correspondingly, AR silencing/knockdown or antagonist (e.g., hydroxyflutamide, enzalutamide) treatment enhanced the cytotoxic effects of cisplatin in bladder cancer cells, even resistant sublines. Enzalutamide was also shown to induce apoptosis and prevent cell migration/invasion in the presence of cisplatin. Mechanistically, enzalutamide treatment was associated with increases in the expression of BAX, cleaved caspase-3, cleaved PARP, and an epithelial marker E-cadherin, and decreases in that of Bcl-2 and mesenchymal markers (e.g., β-catenin, N-cadherin, Slug, vimentin), although there appeared to be no significant differences in their expression between cisplatin + enzalutamide vs. cisplatin or enzalutamide alone.

Similar findings have been reported with other anti-cancer agents. Specifically, AR-positive bladder cancer cells with dihydrotestosterone (DHT) treatment or AR silencing were shown to be less or more, respectively, sensitive to doxorubicin, compared with controls. Additionally, in a gemcitabine-resistant bladder cancer subline, enzalutamide was found to restore its sensitivity while reducing the expression of cyclin D1. A more recent study demonstrated that ASC-J9®, an AR degradation enhancer, could increase sensitivity to not only cisplatin and doxorubicin, but also mitomycin C in AR-positive bladder cancer cells. These findings indicate that activation of AR signaling is associated with chemoresistance in bladder cancer.

We have further explored how AR signals modulate chemosensitivity. There were close correlations of AR expression/activity with those of NF-κB, which is considered to be a key molecule for cisplatin resistance, in bladder cancer cells. We have also found that androgen up-regulates the expression of a *c-fos* proto-oncogene regulator ELK1 in bladder cancer cells and that ELK1 inactivation via stable expression of a shRNA or treatment with a selective α1-blocker silodosin increases sensitivity to cisplatin. Immunohistochemistry in surgical specimens form patients subsequently undergoing cisplatin-based neoadjuvant chemotherapy, phospho-ELK1 positivity was significantly higher in those from non-responders than in those from responders. We recently demonstrated that androgen/AR could down-regulate the expression of BXDC2, which involves ribosome biogenesis, in bladder cancer cells, and loss of BXDC2 in cell lines and surgical specimens was associated with cisplatin resistance. Furthermore, an ERK activator reduced BXDC2 expression in bladder cancer cells, while BXDC2 knockdown failed to affect phospho-ERK expression, suggesting cisplatin resistance via the AR → ERK → BXDC2 signaling pathway.

Similar to our immunohistochemistry data on AR, we recently demonstrated that the rate of ERβ positivity in transurethral resection specimens was significantly lower in responders to cisplatin-based neoadjuvant chemotherapy than in non-responders, especially in female patients. Meanwhile, elevated ERβ expression in adjacent normal bladder tissues was strongly associated with a worse prognosis in patients undergoing cisplatin-based chemotherapy. Two early studies in bladder cancer lines by one group showed that treatment with tamoxifen, along with methotrexate, vinblastine, doxorubicin, cisplatin, mitomycin C, or thiotepa, more strongly inhibited their proliferation, compared to that with each chemotherapeutic drug alone. However, in these assays, the combination effects were not directly compared with that of tamoxifen alone, and it might therefore be unable to conclude that tamoxifen could increase sensitivity to each cytotoxic agent. In an additional in vitro study, gemcitabine combined with tamoxifen showed stronger inhibitory effects on the growth of bladder cancer cells than gemcitabine or tamoxifen alone, but the rates of inhibition by tamoxifen in the absence versus presence of gemcitabine were not directly compared. In a recent study, co-culture of cancer-associated fibroblasts was shown to induce ERβ expression in bladder cancer cells while reducing the cytotoxicity of cisplatin. We further demonstrated that tamoxifen treatment or ERβ knockdown in ERα-negative bladder cancer cells resulted in the enhancement of cisplatin sensitivity. Moreover, in the cisplatin-resistant sublines, ERβ expression was considerably elevated, while E2 induced the expression and activity of β-catenin which was known to involve cisplatin resistance. Thus, activation of ER, especially ERβ, is likely associated with chemoresistance. Additionally, in a study showing that ERα could induce the expression of miR-4324 via binding to its promoter in bladder cancer cells, overexpression of miR-4324 significantly induced the cytotoxic effects of doxorubicin, suggesting an association between ERα activation and increased sensitivity to doxorubicin.

Both androgen and estrogen have been shown to inactivate a tumor suppressor FOXO1 via the AR and ERβ pathways, respectively, in bladder cancer cells. We further found that silencing of FOXO1 or treatment with an FOXO1 inhibitor in bladder cancer cells resulted in the reduction of sensitivity to cisplatin. The expression of an inactivated form phospho-FOXO1 was considerably up-regulated in cisplatin-resistant cells, compared with control cells, and phospho-FOXO1 expression in transurethral resection specimens from patients undergoing cisplatin-based neoadjuvant chemotherapy was more often seen in non-responders than in responders. FOXO1 inactivation could thus be an underlying mechanism for chemoresistance in bladder cancer induced by AR and/or ERβ signals.

A phase 1/1b clinical trial has been conducted to assess if AR modulation enhances the efficacy of chemotherapy (NCT02300610; completed in December 2012). In a total of 10 patients with urothelial cancer receiving standard doses of gemcitabine and cisplatin, oral enzalutamide (80 or 160 mg) was added. Although some of the patients with 160 mg enzalutamide showed partial response, no control arm with no enzalutamide treatment was compared.

2.2. Radiotherapy

We showed that bladder cancer lines endogenously or exogenously expressing a full-length wild-type human AR were significantly less sensitive to irradiation, compared with AR knockdown or control AR-negative sublines, respectively. Correspondingly, DHT or hydroxyflutamide treatment in AR-positive bladder cancer lines significantly reduced or induced, respectively, the cytotoxic effects of irradiation. Meanwhile, radiation-resistant sublines established following 2 Gy ionizing radiation six times/two weeks showed significant elevation in the expression of not only DNA repair genes, such as *ATR*, *CHEK1*, and *PARP-1*, but also AR mRNA/protein. In mouse xenograft models for bladder cancer, considerable increases in radiosensitivity by AR knockdown or anti-androgen treatment were verified. Mechanistically, AR inactivation via knockdown or hydroxyflutamide treatment was found to be associated with a delay in DNA double-strand break repair (e.g., γH2AX resolution) 4–24 h after irradiation. Additionally, in irradiated AR-positive cells, DHT induced the expression of the DNA repair genes, which was restored by hydroxyflutamide. Our findings suggest that AR activity is inversely associated with radiosensitivity in bladder cancer and that concurrent androgen deprivation may function as a sensitizer of irradiation, especially in patients with AR-positive tumor.

In a recent prospective trial (NCT04282876; started in February 2020), patients with muscle-invasive bladder cancer undergoing radiotherapy are being recruited. A group of these patients is randomized to simultaneously receive a gonadotropin-releasing hormone antagonist (i.e., degarelix) as chemical castration. Primary outcome measures include bladder fibrosis 3 months after irradiation, but oncologic outcomes will not appear to be compared.

2.3. Immunotherapy

In an earlier study, DHT was found to inhibit the expression and transactivation of IL-6 induced by BCG treatment in bladder cancer cells. Another study showed that hydroxyflutamide and ASC-J9 increased the expression level of BCG-mediated integrins (e.g., α5β1) and intake of BCG in bladder cancer cells, as well as recruitment of monocytes/macrophages. BCG, along with each AR inhibitor, also more strongly inhibited the growth of bladder cancer cells and chemical carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine-induced bladder tumors in mice, compared with BCG or AR inhibitor alone. These findings implied that AR signaling might contribute to modulating sensitivity to BCG therapy. We then demonstrated direct evidence to indicate the link between AR activation and BCG resistance. AR knockdown or overexpression in bladder cancer lines was associated with considerable induction or reduction, respectively, in intracellular BCG quantity and BCG cytotoxicity. AR expression was considerably higher in BCG-resistant bladder cancer cells following repeating exposure to BCG for over six months, compared with control cells, and AR positivity immunohistochemically determined in non-muscle-invasive bladder cancer specimens from patients who had subsequently undergone intravesical BCG immunotherapy was strongly associated with worse outcomes, compared to those with AR-negative tumor. We also performed DNA microarray screening and identified Rab27b, a small GTPase known to mediate bacterial exocytosis, which was up-regulated in BCG-resistant cells and down-regulated in AR knockdown cells. Indeed, knockdown/overexpression of Rab27b or its known effector SYTL3, as well as treatment with GW4869 known to inhibit Rab27b-dependent secretion, was found to considerably modulate BCG quantity in bladder cancer cells, as well as its cytotoxicity in vitro and in vivo. In addition, Rab27b positivity in the same cohort of patients with BCG therapy was associated with a significantly higher risk of tumor recurrence. Thus, our findings suggest that AR signaling reduces the efficacy of BCG therapy, presumably via modulating Rab27b-induced exocytosis in bladder cancer cells.

The impact of ER signaling on the efficacy of BCG therapy in bladder cancer has also been investigated. In ERα-positive/ERβ-positive bladder cancer cells, E2 reduced BCG attachment and internalization as well as monocyte/macrophage recruitment, whereas tamoxifen and a pure anti-estrogen ICI 182,780 reversed the estrogen effect. These anti-estrogens were also found to enhance the cytotoxic effects of BCG in cell line and mouse models for bladder cancer. In addition, a phase 2 randomized clinical trial is ongoing to assess if genistein, a biologically active isoflavone and a phytoestrogen with structure similar to that of E2, not only helps alleviate the adverse of intravesical BCG therapy but also improves its efficacy (NCT01489813; started in May 2017). In this study, either genistein supplement or placebo is given to the patients with non-muscle-invasive bladder tumor for 10 weeks (i.e., during BCG therapy and one-month post-therapy).

There are a number of PD-1/PD-L1 inhibitors entering clinical trials. Importantly, the efficacy of PD-1/PD-L1 inhibitors, as immune checkpoint inhibitors that attack tumor cells via enhancing the host immune response, is often associated with the levels of PD-L1 expression. In an immunohistochemical study in muscle-invasive bladder cancers, PD-L1 expression was shown to be inversely correlated with the levels of AR expression. We have confirmed this inverse correlation in bladder cancer cell lines. In breast cancer specimens, an inverse correlation of ERα status with *PD-L1* mRNA expression has also been documented.

3. Conclusions

Current evidence indicates a critical role of sex hormone receptor signaling in bladder cancer progression, supporting that urothelial cancer is an endocrine-related neoplasm. However, it remains uncovered how AR and ERs function in urothelial cancer cells. Various studies have also suggested the involvement of sex hormone receptors in modulating sensitivity to conventional non-surgical therapy for bladder cancer. Specifically, activation of AR and ER signals appears to be associated with resistance to chemotherapy, radiotherapy, and BCG immunotherapy, although limited data, especially those on ERα, are available. Accordingly, concurrent inactivation of these, using, for example, anti-AR or anti-ER agents widely used for the treatment of other pathologic conditions such as prostate and breast cancers, is anticipated to improve patient outcomes via sensitizing the efficacy of the conventional therapy, in addition to direct inhibitory effects of androgen/estrogen deprivation. Further investigation of AR and ERs, as well as other molecules directly or indirectly

regulated by AR/ER signals, is required for determining the precise actions of androgens/estrogens in bladder cancer cells, in relation to their impact on modulating sensitivity to conventional therapy, as well as underlying molecular mechanisms for their actions.
