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Pharmacogenomics in bladder cancer

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Abstract

Bladder cancer is a common cancer worldwide. For patients presenting with muscle-invasive disease, the five year survival rate is approximately 50%. Cisplatinum-based combination chemotherapy is recommended in the neoadjuvant setting prior to cystectomy and is also the first line in the metastatic setting. However, the survival benefit of such therapy is modest. The identification of pharmacogenomic biomarkers would enable the rational and personalized treatment of patients by selecting those patients that would benefit most from such therapies sparing others the unnecessary toxicity. Conventional therapies would be recommended for an expected responder while a non-responder would be considered for alternative therapies selected on the basis of the individual's molecular profile. Although few effective bladder cancer therapies have been introduced in the past 30 years, several targeted therapies against the molecular drivers of bladder cancer appear promising. This review summarizes pharmacogenomic biomarkers that require further investigation and/or prospective evaluation, publicly available tools for drug discovery and biomarker identification from *in vitro* data, and targeted agents that have been evaluated in preclinical models.

Introduction

Bladder cancer is the fourth most common cancer in males in the United States and the 6th most common cancer overall [1]. Approximately 20-30% of bladder cancers are diagnosed as muscle invasive (MI) [2], and these patients have a 5-year survival rate of approximately 50% [1]. The remaining 70-80% of bladder cancers are diagnosed as non-muscle invasive (NMI). However, progression to MI disease occurs in ~20% of these patients and progressors have a 5-year survival rate of 43% [3]. Bladder cancer is also one of the most expensive cancers to treat due to lifetime monitoring and treatment that is required [4].

For patients with NMI tumors, the standard of care is transurethral resection of the bladder (TURBT). Intravesical therapy, most commonly Bacillus Calmette-Guerin (BCG) immunotherapy, is also recommended for patients with a high risk of progression. For patients with MI tumors, the standard of care is radical cystectomy and bilateral pelvic lymphadenectomy or radiotherapy. Since, approximately 50% of these patients develop metastases [5], cisplatin-based neoadjuvant combination chemotherapy has been

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recommended based on a response rate of about 38% in the neoadjuvant setting [6] and 50% in the metastatic setting [7] and on a meta-analysis of 11 clinical trials with >3,000 patients that found an absolute survival benefit of 5% at five years [8]. The evidence for survival benefit from adjuvant chemotherapy is controversial because of methodological issues and premature closure of trials [9]. Nevertheless, two meta-analyses found adjuvant chemotherapy led to a 25% reduction in risk of death compared to patients receiving surgery alone [10, 11].

Despite the demonstrated survival improvement from neoadjuvant therapy, its use in the community has been low. Out of >11,000 patients diagnosed with stage III bladder cancer between 1998 -2003, only 1.2% received neoadjuvant therapy [12]. More recently, an analysis of 145 patients with MI tumors (on clinical staging) who received cystectomies between 2003 – 2008 found that 17% received cisplatinum-based neoadjuvant chemotherapy. [13]. However, this reflects practice at a single institution and the true treatment rate may be substantially lower. Low neoadjuvant treatment rates can be attributed, in part, to the reluctance of some physicians to treat patients who will not respond and thus encumber them with toxicity and risk of progression. This situation highlights the need for pharmacogenomic biomarkers to identify patients likely to respond to current (and future) therapies. A *pharmacogenomic* or *predictive* biomarker is a molecule that correlates with drug efficacy or toxicity, and informs on the appropriateness of a proposed therapy for a specific patient. Predicted responders would be assigned the therapy while predicted non-responders would be spared its toxicity and could be assigned to alternative and more promising therapies. In this review, we summarize some of the promising pharmacogenomic biomarkers that have been evaluated in bladder cancer, computational resources to aid in biomarker identification and drug discovery from *in vitro* models, and potential targeted therapies against the genomic drivers of NMI and MI disease. *Prognostic biomarkers* that stratify clinical outcomes independent of treatment have been reviewed recently and will not be discussed [14].

Single gene tumor pharmacogenomic biomarkers

Several single gene pharmacogenomic biomarkers have been evaluated in bladder cancer. In many cases, the predictive value of these biomarkers have been demonstrated in other cancers and the genetic mechanism that modulates chemoresistance in *in vitro* models has been defined. Despite these advances, however, the number of promising single gene predictive biomarkers in bladder cancer is limited. In all cases, prospective evaluation is required before these are utilized in the clinic.

p53

The possibility that the “cellular gatekeeper” p53 is a predictive biomarker is appealing. p53 is a transcription factor whose functions involve DNA repair, cell cycle regulation, and induction of apoptosis [15], and is mutated in >50% of human cancers. Mutation status of p53 has been inferred by immunohistochemical (IHC) detection of nuclear p53, which is detected at high levels when p53 is mutated compared to wild-type p53 which is quickly degraded and absent or present at low levels [16]. Studies have found p53 mutations to be associated with both increased sensitivity and resistance to DNA damaging agents in a variety of tumors [17, 18]. In bladder cancer, a retrospective analysis of p53 in 88 patients receiving cisplatin-based adjuvant chemotherapy in a prospective clinical trial found that the survival benefit was limited to patients with elevated p53 expression by IHC [19]. A phase III trial was implemented to evaluate the predictive value of p53 expression for response to adjuvant chemotherapy [20]. Over 500 patients with pT1/T2N0M0 tumors at cystectomy were enrolled over 8 years and patients with low p53 (≥10% nuclear reactivity based on p53 IHC) were re-consented for randomization to either methotrexate, vinblastine, doxorubicin

(Adriamycin) and cisplatin (MVAC) chemotherapy or observation. Ultimately, p53 status was not able to stratify disease specific survival rates in patients receiving MVAC treatment (P = 0.23). Although disappointing, the trial highlights important challenges in the validation of pharmacogenomic biomarkers in clinical trials. These challenges and others are discussed in the last section of this review.

ERCC1

At least three retrospective studies provide support that the gene excision repair cross-complementing 1 (ERCC1) is a pharmacogenomic marker in bladder cancer patients treated with cisplatinum-based chemotherapy. Cisplatinum treatment triggers the formation of intra-strand crosslink DNA adducts which leads to subsequent cell cycle arrest and apoptosis [21], and repair of DNA adducts via nuclear excision machinery is associated with resistance to platinum-based agents [22]. High expression of ERCC1 is associated with cisplatinum resistance in a variety of cancers [23]. In a cohort of 57 advanced and metastatic bladder cancer patients treated with adjuvant cisplatinum/gemcitabine (GC) or cisplatinum/gemcitabine/paclitaxel (GCT), patients with high levels of ERCC1 (as measured by RT-PCR) had higher survival rates (25.4 versus 15.4 months; P = 0.03) and ERCC1 expression was independent of pretreatment factors such as performance status and age [24]. However, ERCC1 expression was not associated with therapeutic response, suggesting that the clinical value of ERCC1 expression is prognostic, and not predictive. Another study analyzed ERCC1 expression (by RT-PCR) in 108 patients enrolled in a phase III clinical trial (AUO-AB 05/95) where patients with locally advanced tumors were randomized to adjuvant cisplatinum/methotrexate (CM) or methotrexate/vinblastine/epirubicin/cisplatin (M-VEC) chemotherapy [25]. Patients with low expression of ERCC1 had longer survival times (72.4 months vs. 33.1 months) but this was not significant in the Kaplan-Meier analysis (P = 0.19) [26]. Finally, a third study compared the value of ERCC1 expression (assessed by IHC) in patients that received adjuvant GC chemotherapy with patients that did not. In 36 patients that did *not* receive chemotherapy, ERCC1-positive patients had higher overall survival (OS) rates than ERCC1-negative patients (5 year OS of 84.0% vs. 49.2%, P = 0.083). However, the trend was reversed in 57 patients with advanced disease who were treated with GC chemotherapy (5-year OS, 71.8% for ERCC1-negative patients vs. 41.6% for ERCC1-positive patients, P < 0.05 in a multivariate but not univariate analysis). There was also a significant interaction between ERCC1 expression and adjuvant chemotherapy for OS (P = 0.034).

MDR1

The protein *multi-drug resistance gene 1* (MDR1), also known as P-glycoprotein (Pgp) is an ATP-dependent efflux pump that can transport a broad range of substances, including chemical agents such as methotrexate, across the cell membrane. In the 108 patients from the AUO-AB 05/95 trial described above, patients with high MDR1 expression (>75th percentile) had a lower survival rate than patients with low MDR1 expression (<75th percentile) (5 year 0S of 23% vs. 62%, P = 0.0006), and this difference remained significant when the CM and M-VEC treatment arms were analyzed separately (CM arm, P = 0.01; M-VEC arm, P = 0.02) [26].

BRCA1

The breast cancer susceptibility gene 1 (BRCA1) protein is involved in DNA repair, such as that required when DNA is damaged by chemotherapeutics [27]. In 57 patients with MI bladder cancer who received either neoadjuvant GC or cisplatinum/methotrexate/vinblastine (CMV) chemotherapy, patients with low/intermediate BRCA1 expression (measured by RT-PCR) had a favorable prognosis (5-year OS rate of 64%) compared to patients with high

BRCA1 expression (5-year OS rate of 12%, P = 0.002). Furthermore, pathological response rates were higher in patients with low/intermediate levels of BRCA1 expression than in patients with high (66% vs. 22%, P = 0.01) [28]. However, in 57 patients with advanced, surgically incurable bladder cancer who received cisplatinum-based chemotherapy, BRCA1 expression (measured by RT-PCR) was not predictive of overall survival or response to therapy) [24].

Multigene tumor pharmacogenomic markers

Multigene pharmacogenomic biomarkers have been identified from large-scale gene expression profiles such as DNA microarrays. Such markers are identified *de novo* from thousands of genes, without any consideration of their mechanism or known likelihood to modulate chemoresponses. Multigene models are promising because they can more likely capture multiple molecular mechanisms of resistance while being less prone to the technical variability and tumor heterogeneity that lessons the predictive accuracy of single gene biomarkers.

Multigene MVAC signature derived from patients

Takata and colleagues obtained gene expression profiles (~27,000 genes) using cDNA microarrays from 27 patients with MI tumors who were treated with neoadjuvant MVAC chemotherapy [29]. Eighteen samples were used for training to identify a 14 gene signature that discriminated responders (downstaging to ≤pT1) from non-responders (no downstaging; ≥pT2). When applied to an independent test set consisting of the remaining 9 patients (5 responders and 4 non-responders), the signature correctly classified 100% of the responders and 75% of the non-responders. The test set was later expanded to include a total of 22 patients, and the signature reevaluated [30]. The sensitivity (ability to accurately predict responders) was 100% while the specificity (ability to accurately predict non-responders) was 73%. The positive predictive value (proportion of predicted responders that were responders) was 79% while the negative predictive value (proportion of predicted non-responders that were non-responders) was 100%.

Multigene combination chemosensitivity signatures derived from cell lines

The identification of combination chemotherapy pharmacogenomic biomarkers from *in vitro* datasets has two challenges. First, because cell lines are typically screened with single agent compounds, it is vital that single agent pharmacogenomic biomarkers identified from cell lines can be combined to predict responses to combination therapies. Second, because cell lines do not always resemble their tumor counterparts, due to adaptation in culture or cross contamination [31], and because tissue specific biomarkers exist, it is vital that pharmacogenomic biomarkers identified from cell lines be translatable to human tumors in patients.

The ability to predict drug combination chemosensitivity was demonstrated in a panel of bladder cell lines [32]. The single agent sensitivities of cisplatin, paclitaxel, and gemcitabine were determined for 40 human bladder cancer cell lines (BLA-40), which were profiled by DNA microarray (>22,000 genes). For each compound, gene signatures were identified and models generated to calculate a resistance probability. Single drug resistance probabilities were combined to calculate the probability of resistance to combination drug treatments for each pair of drugs. In order to test the combination sensitivity models, fifteen bladder cancer cell lines were randomly selected, and treated simultaneously with each drug pair. The accuracy of the combination chemotherapy models was 80% (P = 0.03) for both the cisplatinum/paclitaxel and gemcitabine/cisplatin combinations, and 73% (P = 0.11) for the paclitaxel/gemcitabine combination.

The ability to translate chemosensitivity signatures across tissue types or between cell lines and patient tumors was demonstrated through an innovative co-expression extrapoloation (COXEN) strategy [33]. Predictive genes for cisplatin and paclitaxel resistance were identified from a panel of 60 cell lines tested by the National Cancer Institute ("NCI-60"; see below). The NCI-60 panel consists of cell lines from 9 different tumor types and does not include bladder. The COXEN algorithm was applied to identify a gene signature of predictive genes that were concordantly expressed between the NCI-60 and BLA-40 cell line panels. Multiple gene expression models were generated for each agent, and these models had average accuracies of 85% for cisplatin and 73% for paclitaxel, in the BLA-40 panel. The COXEN approach was later applied to predict response to combination MVAC chemotherapy in two bladder cancer cohorts (N = 59) which included patients from the Takata cohort [30], described above, and an additional 14 patients with locally advanced or metastatic tumors who received MVAC (Als cohort) [34]. Predictive gene signatures for the individual components of MVAC were identified from the NCI-60 cell line panel, and refined by COXEN to include only predictive genes that were concordantly expressed between the NCI-60 panel and 89 bladder cancer patients who did not receive chemotherapy [35]. When applied to the two testing cohorts, the COXEN-derived combination therapy signature had a sensitivity, specificity, PPV, and NPV of 83%, 64%, 71%, 78%, respectively, at the cutoff value maximizing the Youden index. The COXEN-derived signature also stratified patient outcomes in both the Takata (P = 0.002) and Als (P = 0.015) cohorts [36].

COXEN has also been used to identify multigene pharmacogenomic biomarkers in breast and ovarian cancer patients treated with the single agents or combinations involving doxorubicin, cyclophasphamide, taxol, and 5-FU, based on the NCI-60 cell lines *in vitro* sensitivities to these agents [36]. A clinical trial (clinicaltrials.gov NCT01228942) is ongoing to determine whether the COXEN strategy can predict appropriate treatment therapies for patients with recurrent or persistent ovarian, fallopian tube, or primary peritoneal cancer. In bladder cancer recent NCI/CTEP approval of a proposed SWOG Phase II clinical trial will evaluate the ability of the COXEN strategy to identify responders to neoadjuvant MVAC or GC chemotherapy.

Germline pharmacogenomic biomarkers

Germline polymorphisms are known to influence drug metabolism and can be predictive of drug efficacy and toxicity in cancer. For example, polymorphisms in thiopurine methyltransferase (TPMT) can predict toxicity from mercaptopurine, a therapeutic agent used to treat acute lymphoblastic leukemia [37]. Polymorphisms associated with variation of gemcitabine efficacy and toxicity in cancer have been reviewed previously [38]. However, few germline polymorphisms have been examined in bladder cancer patients. In one study, five polymorphisms in NRAMP1 were evaluated for association with recurrence in patients with superficial tumors who received bacillus Calmette-Guerin (BCG) immunotherapy [39]. Patients with a high risk of recurrence (N = 67) were more likely to have the D543N G:A polymorphism than healthy controls (N = 109; 12% vs. 2%, P = 0.007). All 8 patients with this polymorphism had a recurrence within 6 months, compared to only 1 patient in a group of 37 patients without recurrence (P = 0.027). However, because all patients with recurrent tumors received BCG therapy, it is unclear whether polymorphisms in NRAMP1 were prognostic or predictive. Currently, an ongoing clinical trial (clinicaltrials.gov NCT01206426) is aimed at evaluating whether a set of single nucleotide polymorphisms (SNPs) associated with cisplatin susceptibility is predictive of complete response (downstaging to pT0) in patients receiving neodadjuvant cisplatin-based chemotherapy [40].

Public resources for drug discovery and identification of pharmacogenomic biomarkers

Although the efficacy of any drug or pharmacogenomic biomarker must be demonstrated in patients, *in vitro* cell line models are an appealing high-throughput and cost-effective resource for pharmacogenomics research. In addition to being less time consuming than the analysis of patient tumors, cell line models can easily incorporate newly discovered drugs and combination therapies that have not been examined in patients. Several large and diverse publicly available resources are available that either link the molecular profiles of cell lines with their *in vitro* sensitivities or identify genes targeted by therapeutic compounds (Table 1). Such resources can be mined in order to discover drugs that are likely to be efficacious against tumors with a molecular characteristic of interest, and to identify pharmacogenomic biomarkers for previously screened compounds.

The NCI-60 human tumor cell line screen, managed by the Developmental Therapeutics Program (DTP) at the National Cancer Institute, consists of 60 human tumors from 9 tissues of origin: breast, central nervous system, colon, leukemia, melanoma, non-small cell lung, ovarian, prostate, and renal. Over 100,000 compounds have been screened and drug sensitivity information for over 45,000 compounds is publicly available, including for 93 Food and Drug Administration (FDA)-approved anti-cancer agents [41]. In addition, gene expression profiles, SNP profiles, DNA copy number, and mutation status for select genes in the NCI-60 have been determined (http://dtp.cancer.gov/mtargets/mt_index.html). The COMPARE program identifies compounds whose drug sensitivities correlate with a molecular target of interest, or with the cell line sensitivities of a given compound [42]. These COMPARE analyses enable the identification of drugs that target a population of interest, and the identification of candidate mechanisms for novel compounds [42].

Another tool, the COXEN algorithm described above, was used to identify multigene biomarkers for each of the >45,000 compounds in the NCI-60 database with publicly available drug sensitivity information and to predict *in silico* sensitivities of the BLA-40 cell lines to these compounds. This *in silico* screen identified a compound NSC637993 (6H-Imidazo[4,5,1-de]acridin-6-one, 5-[2-(diethylamino) ethylamino]-8-methoxy-1-methyl-, dihydrochloride), that was found to be a potent inhibitor of the majority of BLA-40 cell lines [33]. Notably, this compound is structurally similar to another top hit from the screen, the topoisomerase II inhibitor C1311, which inhibits growth in a panel of solid tumors and leukemia cell lines [43], and has been evaluated in a phase II trial in breast cancer [44]. These results warrant prospective testing of C1311 in bladder cancer patients.

Two other resources include the Cancer Cell Line Encyclopedia (CCLE), which is a collaboration between the Broad Institute and the Novartis Institutes for Biomedical Research and its Genomics Institute of the Novartis Research Foundation, and The Genomics of Drug Sensitivity in Cancer (GDSC) project, overseen by the Cancer Genome Project at the Wellcome Trust Sanger Institute (UK) and the Center for Molecular Therapeutics and Massachusetts General Hospital Cancer Center (USA). The CCLE contains gene expression profiles, chromosomal copy number, and sequencing data from over 1000 cell lines (including 28 bladder lines). Twenty-four anti-cancer agents (with paclitaxel perhaps the most relevant to bladder cancer) were screened against 494 cell lines, and this information is publicly available [45]. GDSC contains gene expression profiles, gene copy number, and mutation status of commonly mutated genes in over 700 cell lines (including 18 bladder), while screening 138 anti-cancer drugs (including cisplatinum, gemcitabine and methotrexate) [46]. These resources (see weblinks in Table 1) include tools for finding genes or pathways whose expression or mutation status correlates with drug sensitivity.

The Connectivity Map (cmap) is a unique resource that defines functional relationships between drugs and genes [47]. Underlying cmap is a database of gene expression profiles of up to 5 cell lines treated with >1300 perturbagens (chemical or genetic reagents), and corresponding untreated controls. Perturbation profiles are stored that reflect changes in gene expression due to perturbation. For a given gene signature (i.e., a query signature), cmap identifies compounds having perturbagen profiles that are positively or negatively connected to the query signature. One use of cmap is to identify the mechanism of action of a compound, as was done for gedunin, which abrogates AR activity. When queried with a gedunin response signature, cmap identified three heat shock protein 90 (HSP90) inhibitors with high connectivity [47]. Cmap can also be used to identify appropriate combination therapies when the query signature contains pharmacogenomic markers of resistance to a particular compound. For example, when queried with a glucocorticoid dexamethasone resistance signature derived from acute lymphoblastic leukemia cells [48], cmap identified the FDA-approved mammalian target of rapamycin (mTOR) inhibitor sirolimus (rapamycin). Sirolimus treatment reversed glucocorticoid resistance in malignant lymphoid cell lines [47].

Conclusions and challenges in bladder cancer pharmacogenomics

In general, the identification of chemoresponse biomarkers from gene expression data is a challenge. The Microarray Quality Control (MAQC) consortium enlisted 36 independent teams to classify samples from six different microarray datasets with respect to 13 endpoints. Classification accuracy was evaluated using the Matthews Correlation Coefficient (MCC), which ranges from +1 (perfect classification) to -1 (perfect inverse classification), with a value of 0 indicating random classification. Classification of patients based on gender (average MCC ~ 0.95) and estrogen receptor status of breast cancer patients (average MCC ~ 0.70) were relatively easy problems, while classification of breast cancer patients based on response to chemotherapy was substantially more difficult (average MCC ~ 0.30) [49]. This difficulty may be because pharmacogenomic biomarkers are less informative (i.e., have lower fold changes) than biomarkers of other endpoints [50], suggesting that a complex molecular biology drives sensitivity or resistance to therapy. Indeed, multiple mechanisms of chemotherapy resistance are known, including those effecting drug transport and metabolism, and alterations in drug targets [51]. For targeted agents such as trastuzumab, compensation for the target or induction of alternative signaling pathways can lead to resistance [52]. These observations support the use of multigene pharmacogenomic biomarkers such as the Takata and COXEN signatures [30, 36] which await prospective evaluation.

Although several promising pharmacogenomics biomarkers have been identified, their independent validation in prospectively collected patient cohorts is critical [53]. Importantly, as the aforementioned phase III p53 bladder cancer trial demonstrates [20], carefully designing and accruing to such clinical trials is not trivial. Several notable factors in this trial include: 1) The trial assumed an event rate of 50%, but the actual event rate was only 20%, possibly because only low risk patients with pT1-pT2 tumors were enrolled [54]; 2) Only 42% of patients that were p53 positive agreed to randomization, only 67% of those randomized received the full 3 MVAC treatment cycles, and 21% did not receive any chemotherapy; 3) The rate of p53 positive patients (55%) was higher than expected, possibly due to changes in IHC technology during the course of the trial. Although these issues are generally not unique to pharmacogenomics trials, they must be considered when designing trials for pharmacogenomic biomarkers in the future. The successful validation of pharmacogenomic biomarkers will likely depend on careful patient selection, accurate (or conservative) modeling of event rates and patient refusal rates, and robust biomarker assays.

Overall, research in bladder cancer pharmacogenomics has been sobering but promising. Several intriguing single and multigene biomarkers of cisplatin-based therapies have been identified, and additional prospective studies will determine their clinical utility. Importantly, our knowledge about the molecular activity of anti-cancer agents and possible mechanisms of resistance to them are increasing. At the same time, molecular profiles of patient tumors and cell lines are being compiled, along with corresponding information about clinical outcomes and therapeutic responses. Such insights and resources will surely hasten the discovery of novel therapies and pharmacogenomic biomarkers in bladder cancer.

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Table 1

Publicly available pharmacogenomic resources based on *in vitro* data

Database	# Cell lines (# bladder)	Molecular characterization	# drugs screened	Online reference
NCI-60 Human Tumor Cell Line Screen	60 (0)	Gene expression, SNP profiling, DNA copy number, and mutation status for select genes	>45,000	http://dtp.nci.nih.gov/branches/btb/ivclsp.html
Cancer Cell Line Encyclopedia (CCLE)	>1000 (28)	Gene expression profiles, chromosomal copy number, and sequencing data	24 against 494 cell lines	http://www.broadinstitute.org/ccle/home
Genomics of Drug Sensitivity in Cancer (GDSC)	>700 (18)	Gene expression profiles, gene copy number, and mutation status of commonly mutated genes	138	http://www.cancerrxgene.org/
Connectivity Map (cmap)	5 (0)	Gene expression profiles of perturbed cell lines	>1300* against ≤5 cell lines	http://www.broadinstitute.org/cmap/

* includes chemicals and genetic reagents