Genetic determinants for chemo- and radiotherapy resistance in bladder cancer

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Abstract: Bladder cancer (BCa) is burdened by high rates of chemo- and radio-resistance. We reviewed and summarized the current evidence regarding the genetic determinants of resistance in patients treated with chemotherapy and/or radiotherapy (RT) for BCa. Genetic heterogeneity may preexist to treatment arising with tumorigenesis or increasing progressively during the treatment. Several biological pathways seem to be involved in the cellular response to treatment. These pathways comprehend mechanisms leading to modify the intracellular concentration of the drug, mechanisms leading to increase the repair of DNA damage caused by the treatment, mechanisms leading to increase cell survival, despite DNA damage, acting on the signaling pathways affecting apoptosis, mechanisms promoting autophagy. In the present review, we focused on the genetic determinants of resistance affecting the aforementioned mechanisms.

Keywords: Bladder cancer (BCa); urothelial carcinoma; chemoresistance; radioresistance; cisplatin resistance

Introduction

Bladder cancer (BCa) is a burdening maladie with high morbidity and mortality (1). Approximately 25% of cases present with muscle-invasive bladder cancer (MIBC) and 10–15% of patients with metastatic cancer (2). Combination chemotherapy with methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) has been the standard chemotherapeutic (CT) treatment for metastatic urothelial carcinoma since the 1980s (3). More recently, gemcitabine plus cisplatin (GC) demonstrated similar outcomes and lower side effects compared to MVAC in the metastatic setting (4). Furthermore, neoadjuvant platinum-based

CT is adopted into routine clinical practice for patients undergoing radical cystectomy and pelvic lymph node dissection for MIBC (2,5). On the other hand, radiotherapy (RT) with the addition of concurrent radiosensitizing CT can be used as primary organ-preserving treatment and in patients unfit for surgery (6).

Regrettably, a significant proportion of patients experiences disease relapse despite effective therapy and eventually die of tumor metastasis (2,7-9). A poor prognosis has been often attributed to resistance to CT and RT. Genetic heterogeneity seems to contribute substantially to resistance to the treatments (10,11). Changes in the drug uptake and efflux mechanisms, in the DNA damage repair

pathways and in the induction of cell cycle and apoptosis are the most examined biological processes to explain the different clinical response to chemoradiotherapy for several cancers (12-15). However, the genetic mechanisms underlying the molecular processes in BCa inducing resistance to the treatment are still poorly understood.

We sought to review and summarize the current evidence regarding the genetic determinants of resistance in patients treated with CT and/or RT for BCa.

Evidence acquisition

A non-systematic Medline/PubMed literature search was performed with different combinations of terms as "bladder cancer", "chemotherapy", "radiation therapy", "cisplatin", "chemoradiotherapy", "multi-modal therapy", "resistance". Only articles in English language were retained for the analyses. Time period included articles between January 2000 and May 2017. Original articles, reviews, and editorials were selected based on their clinical relevance and additional relevant articles were examined from authors' bibliographies.

Evidence synthesis

General considerations

Resistance to the treatment has been generally categorized into intrinsic and acquired forms (16,17). Tumor heterogeneity seems to play an important role in both models (18).

In the intrinsic forms, tumor initiating potential preexist within the tumor before the treatment (19). According to the stochastic model of cell growth, subpopulations with selective advantageous genetic mutations grow depending on the immune response, microenvironment and intrinsic gene regulatory signals. In contrast, the sensitive clones die due to chemotherapy and are supplanted by resistant cells (20). Cell cycle- and cell adhesion-associated mechanisms represent typical intrinsic forms of resistance.

In the acquired forms, the tumor cells resistance increases developing progressively mutations under the selective pressure of CT and RT (21). In this case, genome evolves from tumor presentation to relapse acquiring sequential mutations that confer an increased drug resistance to the tumor. These cells with enhanced tumor initiating potential, also referred as cancer stem cells, play an integral role in recurrence after CT and RT. Reduced drug accumulation,

increased repair or detoxification, decreased apoptosis and alteration in the drug target site are examples of acquired forms of resistance.

Furthermore, tissue microenvironment has an important role on the response to CT (19,22). In fact, fibroblasts maintain the structural tissue framework and significantly promote tumorigenesis (23,24). Vasculature has an important role on tumor growth and on the distribution of drugs to all tumor cells (25). Immune cells have a direct effect on tumor cells replication or death (26). Moreover, immune cells regulate the extent and the permeability of the vasculature with relevant consequence on drug distribution (27). However, these aspects are still unknown and represent a new hot spot regarding tumor response to treatment.

Biological pathways associated with resistance to platinum-based chemotherapy

Platinum compounds rely their cytotoxic effect on the formation of intrastrand DNA cross-links [mostly double-strand breaks (DSB)], that lead to G2 arrest and apoptosis induction (28). Multiple mechanisms can determine platinum-resistance and can be subdivided into three categories: (I) mechanisms leading to modify the intracellular concentration of the drug (decreased drug uptake, increased drug efflux, intracellular sequestration); (II) mechanisms leading to increase the repair of DNA damage caused by CT; and (III) mechanisms leading to increase cell survival, despite DNA damage, acting on the signaling pathways affecting apoptosis (29).

Biological pathways associated with resistance to RT

Ionizing radiation has a detrimental effect on exposed tissue. Cell damage can be caused by a (I) direct or (II) indirect action of the radiation (30). The direct action occurs when ionizing radiation causes a damage on DNA or the cell wall. An indirect action occurs when ionizing radiation interacts with water molecules resulting in the formation of free radicals. Free radicals can adversely affect various molecules such as nucleic acids, proteins, and lipids, leading to increased oxidative stress. Free radicals can induce DNA DSB as well as single-strand breaks (SSB), abasic sites, base lesions and DNA cross-links. Several mechanisms can induce an altered response to RT, such as pathways related to regulating cell cycle, autophagy promoting the destruction or protection of tumour cells, increased DNA repair, the induction of cell cycle redistribution and

inactivation of apoptosis pathway.

Genetic determinants of decreased drug uptake or increased drug efflux

CTR1 (31), ATP7A (32) and ATP7B (33) genes code for the copper transporter protein and the p-type adenosine triphosphatases proteins responsible for the cellular cisplatin uptake and efflux. The enrichment of the tumor for ATP7A-expressing cells during platinum drug-based treatment of ovarian cancers was significantly associated with worse outcomes (34). By sequencing ATP7A and ATP7B genes in 203 cancer patients, 38 and 61 genetic variations were identified, respectively (35). These genes' expression alterations might be implicated in cisplatin resistance also in BCa cells populations.

The multidrug resistance gene 1 (MDR1) expresses the ATP-dependent cellular efflux pump P-glycoprotein (P-gp) (36). P-gp plays a role in chemoresistance by reducing the intracellular concentration of methotrexate (37), vinblastine (38) and doxorubicin (39), but not of platinum compounds (36). The MDR1 polymorphisms (2677 G>T at exon 21 and 3435 C >T at exon 26) were proved of prognostic significance for disease progression in both small cell lung cancer patients treated with etoposide-cisplatin and in metastatic or recurrent cervical cancer patients treated with cisplatin and ifosfamide, with or without paclitaxel. In patients treated with neoadjuvant MVAC therapy for BCa, the increased MDR1 mRNA and P-gp expression was associated with incomplete response (40).

The canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2 (cMOAT/MRP2) pump is a member of the ATP-binding cassette transporters. The cMOAT/MRP2 pump was overexpressed in several cisplatin-resistant human cancer cell lines with decreased platinum accumulation (41). However, to date, the impact of cMOAT/MRP2 gene overexpression on cisplatin resistance in patients with BCa has not been investigated yet.

Genetic determinants of increased DNA repair

The nucleotide excision repair (NER) is the most important pathway to remove bulky DNA lesions caused by chemotherapeutics, RT and other factors such as ultraviolet light and environmental mutagens (42).

The excision repair cross-complementing group 1 (ERCC1) heterodimerizes with the XPF protein. The ERCC1/XPF endonuclease makes an incision at the 5' end

of the lesion and it is necessary for the further passages of the NER pathway: damaged DNA DSB removal, polymerization, and re-ligation (43). BCa cells were shown to have a significantly higher ERCC1 expression compared to testicular cancer cells and this difference could explain the higher chemoresistance rate in BCa cells (44). Several studies correlated the overexpression of the ERCC1 gene to an increased cisplatin tolerance in BCa cell lines (45,46). The ERCC1 gene is located on chromosome 19q13.2–q13.3. In non-small cell lung cancer (NSCLC), glioma, colorectal cancer, and nasopharyngeal carcinoma two ERCC1 single nucleotide polymorphisms (SNP) were associated with altered chemo and radiation sensitivity of cancer cells: rs11615 (C>T synonymous substitution at codon 118, exon 4, Asn>Asn, C118T) and rs3212986 (C>A substitution in the 30 -untranslated region, C8092A) (47-49). Ma *et al.* reported a significant association between the ERCC1 codon C118T polymorphism and the response rate in patients with T4 BCa treated with platinum-based chemotherapy. It was suggested that the ERCC1 single nucleotide polymorphisms might have an effect on ERCC1 mRNA expression (50). Bellmunt *et al.* reported for the first time that, in advanced BCa setting, patients with high mRNA level of ERCC1 had a worse prognosis after treatment with cisplatin-based CT compared to those patients with low mRNA level of ERCC1 (51). These results were confirmed by Hoffman and colleagues, who analyzed the tumor samples from 108 patients with locally advanced BCa and treated with cisplatin-based CT (52). The high ERCC1 gene expression was an independent predictive factor of worse overall survival and significantly correlated with lower progression-free survival (52). Similarly, the ERCC1 expression seems to be associated with a higher sensitivity to the radiation therapy. In fact, among four different BCa cell lines examined, the cell line with the highest ERCC1 expression had the highest resistance to RT exposure (45). Ahmad *et al.* reported that ERCC1-deficient cells were more sensitive to ionizing RT exposure (43). Furthermore, in a retrospective analysis of 78 patients who underwent chemoradiation therapy for muscle invasive and severe high-risk BCa the rs13181 SNP of the ERCC1 gene together with an XRCC1 mutation was an independent predictor of better cancer-specific survival (53).

The excision repair cross-complementing group 2 (ERCC2) or xeroderma pigmentosum group D (XPD) is a helicase with a key role in gene transcription and in the NER pathway. In human cell lines, a loss-of-function of the ERCC2/XPD gene was correlated with cisplatin sensitivity,

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whereas an overexpression with cisplatin resistance (53,54). The ERCC2 gene is located on chromosome 19q13.32. The rs13181 (A>C substitution at codon 751, exon 23, Lys>Gln), the rs1799793 (G>A substitution at codon 312, exon 10, Asp>Asn) and the rs238406 (C>A substitution at codon 156, exon 6, Arg>Arg) are the most important SNPs examined to predict chemoresistance. Moreover, the somatic ERCC2/XPD mutations were found to be associated with a pathologic complete response to neoadjuvant cisplatin-based CT in muscle-invasive urothelial carcinoma (55,56).

The XPC-HR23B complex is responsible for binding of DNA adducts and is an intermediate signaling protein for the cell cycle checkpoint control and apoptosis after DNA damage in the NER pathway (57). The xenoderma pigmentosum group C (XPC) gene is located on chromosome 3p25.1. An overexpression of the XPC protein resulted in an increased sensitivity and apoptotic cell death of BCa cell lines after cisplatin treatment (58). The frequency of the variant allele for A/C polymorphism (A>C substitution at codon 939, exon 15, Lys>Gln) was found to be significantly higher in the BCa cases compared to the controls (59). Several studies investigated the association between XPC poly (AT) deletion/insertion (PAT –/+) polymorphism and cancer susceptibility (60,61). An epigenetic mechanism histone-mediated was recently associated with XPC silencing in BCa (62). Although this mechanism was not implicated in chemo resistance, it was correlated with cancer development and severity (62).

The mismatch repair (MMR) is a highly conserved, strand-specific repair pathway which recognizes DNA damage induced by platinum compounds. Defects in MMR can be inherited, as in the case of hereditary nonpolyposis colorectal carcinoma, or can occur after epigenetic silencing as demonstrated in ovarian, endometrial, gastric, and colorectal carcinoma (63-65). In most of these cancers, a defect of the MMR was caused by a downregulation of the hMLH1 and hMLH2 genes resulting in a cisplatin-resistance (66). In BCa cell lines a reduced expression of the hMLH1 and hMLH2 genes was associated with a higher rate of muscle-invasive disease. However, evidence regarding the impact of downregulation of the MMR pathway on sensibility to chemo and RT in BCa cell lines is still lacking.

Other pathways seem to play a role in DSB repair after DNA damage secondary to chemo and RT. The Mre11/Rad50/Nbs1 (MRN) complex is involved in homologous recombination and non-homologous end joining pathways. The disruption of the MRN complex using an adenoviral vector containing a mutant dominant negative RAD50 was associated with an increase in cisplatin sensitivity in human squamous cell carcinoma cells (67). However, to our knowledge, the role of other DSB repair pathways in BCa has not been investigated yet.

The base excision repair (BER) pathway is responsible for the SSR caused prevalently by ionizing radiation. The X-ray repair cross-complementing group 1 (XRCC1) and apurinic/apyrimidinic endonuclease 1 (APE1) are the most investigated proteins of the BER pathway. The XRCC1 is a scaffolding protein that interacts with the ligase III and several polymerases (68). The XRCC1 gene is located on chromosome 19q13.2-13.3. The rs1799782 (codon 194, exon 6, Arg>Trp), the rs1799793 (codon 312, exon 10, Arg>His) and the rs25487 (codon 399, exon 10, Arg>Gln) are the most important SNPs leads to amino acid substitutions. The XRCC1 has been extensively examined as a prognostic factor of oncologic outcomes in patients with NSCLC. A recent meta-analysis considering 4,807 patients, genetic SNPs in the XRCC1 gene were significantly correlated with higher sensitivity to platinum-based CT in patients with NSCLC (69). Evidence in BCa is scarce. Sakano *et al.* in the over mentioned study, reported that the rs25487 SNP together with a positive expression for ERCC1 was independently associated with a longer cancer-specific survival (53).

The High mobility group box protein 1 (HMGB1) is a transcriptional protein that acts as a damage recognition enhancer and as a DNA remodeler in several reparation processes (70). HMGB1 also interacts with several transcription factors such as p53, p73, the retinoblastoma protein and members of the Rel/NF-kB family (70). HMGB1 is implicated in several other processes such as evasion of programmed cell death and growth signaling. HMGB1 plays paradoxical roles, as it was associated with a worse prognosis in several cancers when overexpressed, while its suppression inhibited autophagy and increases apoptosis enhancing the anticancer treatment effectiveness (71). The HMGB1 was found to mediate autophagy during chemotherapy in several cancers (72). *In vitro* experiments in BCa cell lines indicated a positive correlation between higher levels of HMGB1 protein and resistance to radiation in various BCa cell lines (68). In *in vivo* experiments conducted on mouse HMGB1-knockdown, BCa tumor xenograft showed a significantly higher response to RT (73). In an analysis of 164 patients newly diagnosed with BCa, HMGB1 overexpression was independently associated with shorter disease-free survival and overall survival in patients with BCa (74). A recent

study suggested that suppressing HMGB1 expression with a small interfering RNA attenuate gemcitabine-induced autophagy and increase apoptosis blocking ERK and JNK activation and bcl-2 phosphorylation (75). Indeed, new drugs targeting this pathway might improve the anticancer efficacy of gemcitabine against BCa.

Genetic determinants of signaling pathways affecting apoptosis

The CT and RT have a cytotoxic effect exerted by the initiation of apoptotic pathways in several types of cells (76). The bcl-2 family is constituted by several proteins with pro- and anti-apoptotic function and is responsible for the control the release of cytochrome C from the mitochondria. The permeabilization of mitochondrial membranes induces the activation of the intrinsic apoptotic pathway. In cell culture models, the antiapoptotic bcl-2 family proteins played a role in suppressing cell death induced by cytotoxic anticancer drugs (77). In T24 resistant BCa cell lines, it was demonstrated that overexpressed bcl-2 protein inhibits cisplatin-induced Bax translocation and the subsequential downstream intrinsic apoptotic pathway (78). Conversely, downregulation of Bcl-2 by small interfering RNA induced Bax and cytochrome c redistribution and reverse cisplatin-resistance (78). In the same cell lines, cisplatin and gamma-irradiation seemed to induce a significant expression of Bfl-1/A1 (a bcl-2 family member) by the nuclear factor-kappaB (NF-kappaB) compared to parental cells. These results suggest that Bfl-1/A1 might play a crucial role in the suppression of the apoptosis Bfl-1/A1 by TNF-α, p53, B cell antigen receptor ligation and Bax and induce resistance to chemotherapeutics. The BCL2-938C>A (rs2279115) and BAX-248G>A (rs4645878) promoter region SNPs were associated with poor progression-free survival in patients with NSCLC (79). To our knowledge, no study reported polymorphisms of the bcl-2 family protein associated with worse prognosis in patients with BCa.

The P53 gene is the most frequently mutated gene (>50%) in human cancer. Upon DNA damage or other stresses, various pathways can induce p53 activation leading to a cell cycle arrest to allow repair and survival of the cell or apoptosis to discard the damaged cell. The p53 protein plays a role in apoptosis, genomic stability, and inhibition of angiogenesis. The p53 gene is located in 17p13.1 and encodes at least 15 protein isoforms. Experiments on BCa cell lines lacking functional p53 showed that tumor cells were more resistant to cisplatin (80). The reconstitution of

wild-type p53 could be a possible strategy for reversing the antiapoptotic phenotype and restoring chemosensitivity (81). Initial promising results suggested that patients with altered p53 in the tumor have a higher sensitivity to cisplatin-based adjuvant CT (82,83). A more recent randomized clinical trial failed to confirm the prognostic value of p53 in patients, although the high patient refusal rate and failures to receive assigned therapy severely compromised the power of the study (84).

The extrinsic apoptosis pathways are activated through the transduction of extracellular signals. Death receptors are members of the tumor necrosis factor (TNF) receptor gene superfamily and induce apoptosis excluding the bcl-2 superfamily members (85). The most important receptors inducing the extrinsic apoptosis pathway are CD95 (for CD95L), TNFR1 (for TNF-α and lymphotoxin-a) and TRAILR1 and TRAILR2 (for TRAIL). TRAIL (TNF-related apoptosis-inducing ligand) is a type II transmembrane protein, that induces apoptosis in a wide variety of transformed cell lines. Four homologous receptors for TRAIL (TRAIL-R) were identified. TRAIL-R1 and -R2 contain intracytoplasmic death domains and mediate apoptosis *in vitro*. In contrast, TRAIL-R3 and TRAIL-R4 do not mediate apoptosis (86-89). In BCa cell lines, promoting the overexpression of TRAIL with leucine zipper forms led to a higher cytotoxic effect of cisplatin (90). This result was confirmed even in resistant BCa cells (91). Furthermore, replicative adenovirus armed with TRAIL synergistically enhanced the antitumor effect of gemcitabine growth inhibitory effects of gemcitabine, accompanied by increased apoptosis in T24 BCa cell lines (92). In a study group of 91 BCa patients, C1595T (rs1131580) and DR4 C626G (rs4871857) of TRAIL gene SNPs were identified (93). The rs1131580 SNP was significantly lower in patients with BCa compared to controls, while the rs4871857 SNP was significantly increased in high-grade BCa patients compared to those with low-grade BCa.

Conclusions

A significant proportion of patients with BCa experiences recurrence after CT and RT. In the last decade, the acquired knowledge of the biological pathways involved in the cellular response to treatment has led to the publication of several studies analyzing the genes polymorphisms related to an altered response to platinum-based CT and RT in cancer cells. Genetic heterogeneity may preexist to treatment arising with tumorigenesis or increasing

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progressively during the treatment. The mechanisms related to chemo- and radio-resistance affect many pathways such as those involved in drug absorption and efflux, in DNA damage repair, in cell cycle and apoptosis. Gene mutation or altered expression of the proteins that are key components of these pathways seem to be associated with the response to the treatment. A greater awareness of the inherited genetic differences in drug metabolic processes will pave the way for multimodality treatment strategies utilizing different target signaling pathways.

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Footnote

Conflicts of Interest: I certify that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript are the following: Shahrokh F. Shariat owns or co-owns the following patents: Methods to determine prognosis after therapy for prostate cancer. Granted 2002-09-06. Methods to determine prognosis after therapy for bladder cancer. Granted 2003-06-19. Prognostic methods for patients with prostatic disease. Granted 2004-08-05. Soluble Fas: urinary marker for the detection of bladder transitional cell carcinoma. Granted 2010-07-20. He is advisory board member of Astellas, Cepheid, Ipsen, Jansen, Lilly, Olympus, Pfizer, Pierre Fabre, Sanofi, Wolff. He is speaker for Astellas, Ipsen, Jansen, Lilly, Olympus, Pfizer, Pierre Fabre, Sanochemia, Sanofi, Wolff. The other authors have no conflicts of interest to declare.

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