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Platinum Priority – Editorial

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MSH2 Expression and Resistance to Cisplatin in Muscle-invasive Bladder Cancer: A Mix of Progress and Challenges

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Bladder cancer is tenth most common cancer worldwide, and the sixth most frequent malignancy among men and the ninth leading cause of cancer-related death [1]. Treatment of its most aggressive form—muscle-invasive bladder cancer (MIBC)—relies on radical cystectomy complemented with chemotherapy in a neoadjuvant or adjuvant setting, with MVAC (methotrexate, vinblastine, doxorubicin, cisplatin) or GC (gemcitabine, cisplatin) constituting the mainstays [2]. Nonetheless, the response rate to cisplatin-based regimens does not exceed 50% [3] and identification of cisplatin-resistant MIBC remains a challenge. Thus, predictive biomarkers that allow early identification of these cases and redirection of patients to alternative regimens are urgently needed.

In this issue of *European Urology*, Goodspeed and colleagues [4] report on low *MSH2* expression as a potential biomarker to predict resistance to cisplatin-based therapy in MIBC. Starting from a whole-genome CRISPR screen, the first ever performed to find mediators of cisplatin-resistance in bladder cancer cell lines, the authors identified *MSH2* (along with *MLH1*) as the best candidate gene. Despite being a laborious technique with extensive bioinformatics analysis, the CRISPR screen allows complete gene knockdown and simultaneous assessment of multiple genes, with higher specificity compared to other methodologies [5]. In vitro validation of this finding was carried out via *MSH2* gene knockdown in cancer cell lines and assessment of caspase and DNA-damage response. Associations emerged between cisplatin resistance and decreased apoptosis, and between *MSH2* downregulation and reduced DNA-damage response. Interestingly, this *MSH2*-related mechanism was specific for cisplatin, as cells with lower MSH2 expression remained sensitive to the other drugs included in standard MVAC and GC regimens, as well as to oxaliplatin, a drug from the cisplatin family that is often used as an alternative for MIBC patients refractory to cisplatin-based chemotherapy. Remarkably, oxaliplatin cytotoxicity mostly depends on DNA cross-linking, which inhibits DNA synthesis and transcription, whereas cisplatin (and carboplatin) cytotoxicity additionally relies on addition of alkyl groups to DNA, resulting in DNA fragmentation by repair enzymes to replace alkylated bases. This may explain the dissimilar behavior of cells with MSH2 downregulation exposed to cisplatin or oxaliplatin, as well as the association with impaired DNA damage repair.

The obvious next step was to validate these findings in vivo, which was accomplished in the study via analysis of the bladder cancer data set from The Cancer Genome Atlas (TCGA) [4]. In this MIBC patients cohort, *MSH2* loss was not associated with standard clinicopathological features, and *MSH2* transcript levels only weakly correlated with protein expression, suggesting a post-transcriptional regulatory mechanism. Intriguingly, low MSH2 levels were not associated with a greater number of neoantigens in MIBC, a finding that is in line with the paucity of microsatellite instability in bladder cancer, despite frequent *MLH1* and *MSH2* downregulation (but not loss). Thus, the molecular mechanisms underlying the contribution of altered MLH1 and MSH2 expression for bladder carcinogenesis require further clarification. Furthermore, in the TCGA data set,

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most MIBC cases with low MSH2 expression had progressive disease and worse overall survival on platinum-based treatment when compared to those with high or intermediate MSH2 levels. However, MSH2 levels did not predict overall survival in patients who did not receive chemotherapy or radiation-based therapy. Importantly, these findings were replicated in the subset of high-risk patients with lymph node metastases and high stage disease. These findings clearly suggest that MSH2 downregulation is involved in cisplatin resistance across the clinical and pathological spectrum of MIBC. Remarkably, in testicular germ cell tumors, *MSH2* loss was also associated with resistance to cisplatin [6], and in advanced-stage sporadic colorectal cancer, loss of mismatch repair genes (including *MSH2*) predicted impaired response to adjuvant chemotherapy [7]. In this context, one of the main limitations of the study by Goodspeed et al. [4] is the absence of a MIBC patient cohort with biological samples (tissue or urine) that could be interrogated for *MSH2* expression (transcripts and protein) and its association with therapy and patient outcome. Indeed, translation of these findings into clinical practice will require a correspondence between MSH2 protein expression levels determined via reverse-phase protein array, which are the only TCGA data available, and immunohistochemistry, which is the proteomics technique most widely used (and almost universally available) in routine pathological practice.

The prognostic value of MSH2 downregulation among MIBC patients treated with platin-based therapy could be similar to the prediction of urothelial carcinoma development in Lynch syndrome (LS). Whereas LS is an established risk factor for the development of upper urinary tract cancer, data for bladder cancer remain controversial, although *MSH2* mutation carriers seem to have a higher risk of bladder cancer [8]. In Danish LS families, *MSH2* mutations were more frequently found in bladder cancer patients, and these should be prioritized for surveillance for bladder and upper urinary tract cancer [8]. Thus, it would be interesting to compare the results of platinum-based treatment of invasive urothelial carcinoma in LS and sporadic cancer patients. This might provide additional clues about the clinical usefulness of MSH2 expression for predicting response to cisplatin.

Although the pioneer results reported by Goodspeed et al seem promising, unravelling a potential biomarker predictive of resistance to cisplatin will require further clinical validation not only to establish a method for evaluating MSH2 expression for reproducible use in routine practice but also to standardize patient eligibility, clinicopathological characteristics, and methods for assessing response to therapy. Furthermore, considering that other molecular alterations such as *ERCC2* [9] and *ERBB2* [10] mutations might also convey predictive information concerning sensitivity to cisplatin, assembly of a combined biomarker panel might constitute a valuable ancillary tool for selecting MIBC patients for chemotherapy regimens, and could facilitate early identification of cisplatin-refractory disease and direction of patients to oxaliplatin-based regimens and thus improve patient outcomes and quality of life.

Conflicts of interest: The authors have nothing to disclose.

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