Muscle-Invasive Bladder Cancer

Subjects: Oncology

Contributor: Anastasia Hepburn

Bladder cancer is the tenth commonest cancer worldwide. The occurrence of bladder cancer is a complex, multi-factor, multi-step pathological process, which has both internal genetic factors and external environmental factors. Bladder cancer can be divided into non-muscle invasive bladder cancer and muscle invasive bladder cancer. Clinically, most patients with bladder cancer are in well-differentiated or moderately differentiated non-muscular invasive bladder cancer at the time of diagnosis (about 10% of them eventually develop into muscular invasive bladder cancer or metastatic bladder cancer)

muscle invasive bladder cancer | neoadjuvant chemotherapy | cisplatin | biomarker | machine learning | CNGB1

1. Introduction

Bladder cancer is the tenth commonest cancer worldwide with 549,000 new cases and 199,000 deaths reported in 2018 [1]. While 85% of patients present with less aggressive non-muscle invasive bladder cancer (NMIBC), they have a high risk of recurrence (50–70%) and up to 25% will progress to more advanced disease [2]. For patients that present with, or progress to, muscle invasive bladder cancer (MIBC), the mainstay of treatment is radical cystectomy and radiotherapy [3]. However, 5-year disease-free survival is as low as 15–35% and up to 50% of patients develop metastasis within two years of surgery inevitably succumbing to their disease [4]. Failure is usually due to occult micrometastatic disease present at diagnosis. Cisplatin-based neoadjuvant chemotherapy (NAC), including administering regimens such as MVAC (Methotrexate, Vinblastine, Doxorubicin and Cisplatin), is a promising strategy to achieve pathological downstaging as well as early eradication of micrometastasis to improve patient survival [3].

High level evidence from two large, randomised trials and two meta-analyses demonstrated that the MVAC regimen prior to cystectomy resulted in a 5–10% increase in 5-year cancer-specific survival (CSS) in comparison to cystectomy alone [5][6][7][8]. Interestingly, the 5-year CSS for responders to NAC is 90% in contrast to 30–40% for non-responders. However, only approximately 40% of patients will have a major response to NAC (defined as absence of muscle-invasive disease and lymph node metastasis; <pT2 and pN0) and benefit from it. Furthermore, non-responders suffer substantial overtreatment, delay of surgery and loss of opportunity for further therapy due to physical deterioration from toxicity or to disease progression. Therefore, identification of a reliable method to stratify NAC administration based on predicted response is of critical importance for the management of MIBC patients and may ultimately lead to future personalised medicine. Recent studies have explored DNA repair gene

mutations (*ERCC1*, *ERCC2*, *BRCA1*) [9][10][11][12], regulators of apoptosis (survivin, Bcl-xL) [13], receptor tyrosine kinase mutations (*ERBB2*) [14], gene expression signatures [13][15][16][17][18][19], molecular subtypes of bladder cancer [20][21][22][23] and alterations in the cellular mechanisms of drug uptake/transport (CTR-1, MDR1) [24][25] as potential predictors of response to NAC and offer promise for improving patient selection for such treatment and clinical outcomes. However, to date, no biomarker exists in the clinical setting to prospectively identify the patients most likely to benefit from NAC.

2. CNGB1 as a Predictor of Muscle-Invasive Bladder

To date, there are no clinically approved biomarkers predictive of response to NAC and identification of such predictors remains crucial for the selection of the most effective treatment for MIBC patients. To address this clinical urgency, a mutli-methods analysis approach of differential gene expression and machine learning methods was undertaken on a cohort of MIBC patients highly selected for an exquisitely strong chemotherapeutic response or marked resistance and/or progression. This approach identified a 9-gene signature able to select responders from non-responders with 100% accuracy which further showed significant association with survival in our limited external validation and also highlighted *CNGB1* as a promising potential biomarker to predict chemoresponsiveness of MIBC patients through validation in internal and external patient cohorts as well as in vitro studies.

Multiple signatures predicting response to NAC have been reported previously in MIBC, identifying markers such as *survivin*, *IPO7*, *TOP2A*, *PIR51*, *RACGAP1* and solute carriers such as *SLC16A3* and *SLC22A18* [13][15][16][17][18]. Even though none have been incorporated into routine clinical practice so far, they have provided positive steps towards achieving a precision medicine approach for the treatment of this disease. The interplay between bladder tumour biology, chemotherapy response and resistance mechanisms are complex. Recently, it has been suggested molecular subtyping may impact patient benefit to NAC [20][21][22][23]. Molecular subtypes have been discovered that are associated with specific clinicopathological characteristics and differential sensitivity to treatments. It will be interesting to interrogate expression of our signature and *CNGB1* in these subtypes particularly those expected to be resistant to NAC, such as the p53-like expression subtype.

Our analysis led to the identification of *CNGB1*, which encodes for one of the subunits that compose cyclic nucleotide-gated channels (CNGs). CNGs belong to the superfamily of voltage-gated ion channels and are key components for signal transduction by controlling the influx of cations, including Ca^{2+} ions, in response to signal-induced changes of cGMP or cAMP levels [26]. CNGs were first identified in retinal photoreceptors and olfactory sensory neurons, in which their function has been extensively studied [27][28]. Interestingly, Olfactory receptor 5P3 (*OR5P3*), a G-protein coupled-receptor also expressed by olfactory receptor neurons, was similarly upregulated in ‘non-responders’ and also identified in Signature 2 by RGIFE. CNGs expression has also been seen in other tissues including brain, liver and kidney, though their function in non-sensory cells is not as well understood [29]. Recently, a clinically aggressive variant of bladder cancer, sarcomatoid carcinoma, was shown to carry frequent mutations of *CNGB1* [30]. CNGs form heterotetramers composed of up to three different types of subunits that determine the channel’s functional features, including *CNGA1* which was also observed to be upregulated by ‘non-

responders' [31]. For example, rod photoreceptors comprise three CNGA1 subunit and one CNGB1 subunit, with the latter conferring Ca^{2+}/Calmodulin-dependent modulation of channel activity. Upregulation of calcium-sensing proteins *CALML3*, *CALML5* and *S100A7A* was also noted in 'non-responders'. Previously, a correlation of S100A7A (also known as psoriasin) expression with poor bladder cancer survival was seen [32]. A role for other S100 family of calcium-binding proteins in bladder cancer cisplatin sensitivity has also been reported [33][34]. Interestingly, *HIST1H4F*, identified by both RGIFE and differential gene expression analysis, has been shown previously to be part of a prognostic signature [35].

Calcium ions are one of the most important cellular messengers in biology and have been implicated many hallmarks of cancer [36]. Several drugs have been reported to block CNG channels including calcium channel blockers currently used in clinical practice in the management of high blood pressure, angina and cardiac arrhythmias, including dihydropyridines (e.g., nifedipine), phenylalkylamines (e.g., verapamil) and benzothiazepines (e.g., diltiazem) as well as the local anaesthetic tetracaine and calmodulin antagonists [27][37][38]. Calcium channel blockers can also enhance chemotherapy cytotoxicity by blocking the multidrug resistance protein P-glycoprotein, which through its function as an adenosine triphosphate-dependent drug efflux pump reduces intracellular chemotherapeutic drug accumulation [39].

3. Conclusions

We identified a novel gene signature able to select responders from non-responders with high predictive accuracy and highlighted CNGB1 as a simpler proxy as a promising potential biomarker to predict chemoresponsiveness of MIBC patients. Our signature gene set and the role of CNGB1 as a simpler proxy warrants additional larger scale validation in similar established cohorts of patients stratified based on response to NAC and ideally in trials of biomarker directed therapy in improving survival for MIBC patients.

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