The Role of Vitamin D Receptor Polymorphisms in Predicting the Response to Therapy in Nonmuscle Invasive Bladder Carcinoma

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Purpose: Clinical and pathological predictors of bladder carcinoma recurrence and progression are relatively well defined. However, there is a paucity of genetic data specifically on the association of single nucleotide polymorphisms in specific genes for predicting recurrence and progression following immunotherapy. The VDR gene was found to regulate the immunomodulatory effects of vitamin D and it enhances the innate immunity system. We evaluated 3 VDR single nucleotide polymorphisms and their predictive role on the response to immunotherapy.

Materials and Methods: Patients with bladder cancer at intermediate-high risk who underwent post-transurethral resection intravesical bacillus Calmette-Guérin in Singapore and Hong Kong from 1995 to 2014 were recruited for analysis. We evaluated 3 VDR single nucleotide polymorphisms using polymerase chain reaction. Kaplan-Meier survival curves and relationships with outcomes were analyzed by multivariable Cox regression.

Results: A total of 338 predominantly Chinese patients were included in study. Individuals carrying the VDR genotype Bsm A/G were significantly associated with lower time to recurrence after bacillus Calmette-Guérin therapy ($p < 0.001$). On multivariable analysis the HR of recurrence in patients with the Bsm A allele was 3.95 times that in patients without the allele ($p = 0.037$). Patients with the VDR GATC subhaplotype were 3.05 times more likely than patients with other subhaplotypes to experience recurrences ($p = 0.003$). Study limitations include the small sample size and the lack of information on previous bacillus Calmette-Guérin vaccine exposure and on vitamin D levels.

Conclusions: Our findings in this study suggest that various VDR single nucleotide polymorphisms are associated with recurrences after bacillus Calmette-Guérin immunotherapy. Further functional studies should be performed to elucidate the significance of the VDR gene in the management of bladder cancer and the potential therapy implications.

Key Words: urinary bladder neoplasms; carcinoma; polymorphism, single nucleotide; receptors, calcitriol; BCG vaccine

Abbreviations and Acronyms

1,25-VD = activated vitamin D
BCG = bacillus Calmette-Guérin
NMIBC = nonmuscle invasive bladder cancer
PCR = polymerase chain reaction
SNP = single nucleotide polymorphism
TLR = Toll-like receptor
VDR = vitamin D receptor

Accepted for publication May 22, 2018.

No direct or indirect commercial incentive associated with publishing this article.

The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

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BLADDER cancer is the most common cancer of the urinary system and the ninth most common cancer around the world.[1] Approximately 70% of all patients with bladder cancer present with nonmuscle invasive tumors at first diagnosis. Due to its prevalence, high recurrence rates and cystoscopy

0022-5347/18/2004-0001/0
THE JOURNAL OF UROLOGY®

https://doi.org/10.1016/j.juro.2018.05.120
Vol. 200, 1-6, October 2018
Printed in U.S.A.

dependent surveillance the treatment cost of NMIBC accounts for more than 60% of the total treatment cost of all patients with bladder cancer.[2] Bladder cancer care accounts for $3.98 billion of the annual medical expenditure in the United States[3] and the economic burden of this condition on the individual is significant. As reported by Avritcher et al the lifetime expenditure on treatment of patients whose course follows the best possible bladder cancer outcomes was $120,684.[4]

Although transurethral resection of bladder tumor followed by intravesical instillation of BCG is the standard of care for intermediate to high risk bladder cancer, recurrence rates are documented to be as high as 80%.[5] Management of recurrences has accounted for approximately 60% of this lifetime cost.[6]

It was hypothesized that intravesical BCG therapy triggers local immune responses that involve CD4+ T cells, macrophages, interferon-γ, urinary cytokines and tissue necrosis factors.[7,8] These local immune responses persist for months and appear to correlate with antitumor activity. The VDR gene was found to regulate the immunomodulatory effects of vitamin D and enhance the efficacy of pulmonary tuberculosis treatment.[9,10] Upon binding to 1,25-VD the vitamin D-nuclear VDR complex upregulates the innate immunity system, resulting in increased production of cathelicidin, an essential component of the host immune response.[10]

Currently clinical and pathological factors predicting recurrence and progression after BCG treatment of nonmuscle invasive bladder carcinoma are relatively well defined. Numerous studies have suggested that VDR polymorphism has a significant role in tumorigenesis.[11] However, the association of SNPs in specific genes to predict recurrence and progression following BCG treatment in the context of bladder carcinoma has been lacking. In this study we hypothesized that these immunomodulatory effects of VDR signaling would have a predictive role in individual responses to immunotherapy.

PATIENTS AND METHODS

We evaluated the records of 338 predominantly Chinese patients, comprising 148 healthy controls recruited from the university health and wellness center, and 190 patients with bladder cancer recruited from National University Hospital of Singapore and Chinese University of Hong Kong. The 190 patients with NMIBC underwent post-transurethral resection intravesical regimens of BCG or BCG with interferon-α at 1 of 2 tertiary hospitals in Singapore and Hong Kong from 1995 to 2014. Patients with bladder carcinoma deemed at intermediate to high risk according to the EORTC (European Organisation for Research and Treatment of Cancer) risk classification were selected. Sociodemographics, clinical data and peripheral blood were prospectively obtained at the time of initial treatment and before BCG treatment, and then stored.

Repeat transurethral resection of bladder tumor became a routine clinical practice after its inclusion in the 2008 EAU (European Association of Urology) guidelines committee for the treatment of nonmuscle invasive bladder cancer. Thus, after 2008 all patients underwent routine repeat transurethral resection of bladder tumor. Patients received the 6 BCG induction doses and at least 3 maintenance BCG doses. For the initial intravesical BCG regimen the Connaught strain was administered. The Tokyo strain was adopted from 2015 and thereafter due to the worldwide shortage.

Study inclusion criteria were patients with completely resected, histologically proven intermediate or high risk nonmuscle invasive urothelial carcinoma of the bladder who were scheduled for intravesical BCG therapy. Patients who have received prior intravesical therapy other than BCG were eligible provided that they have not received any other intravesical agent within 3 months before study inclusion. Exclusion criteria included immunosuppression, concurrent urothelial carcinoma of the upper urinary tract or active tuberculosis.

Regular surveillance after completion of the BCG regimen was performed with cystoscopy and cytology every 3 to 6 months according to the EAU guidelines. In all documented cases of recurrence bladder biopsies were done. Controls were recruited from the university wellness center when they presented for health screening. All participants agreed to the use of biological samples, including routine urine dipstick analysis, for study purposes. The study was approved by the local ethics committees of Singapore and Hong Kong. Written informed consent was also obtained to use patient data.

Vitamin D Receptor Genotyping

Three VDR SNPs, including Taq(rs731236), Bsm(rs1544410) and Fok(rs10735810), which have commonly been implicated in susceptibility to tuberculosis infections, were evaluated by PCR, followed by high resolution melt analysis and DNA sequencing. PCR was performed using the real-time Rotor-Gene® Q PCR cycler. Nucleic acid was extracted from whole blood using the QIAamp® DSP DNA Mini Kit (Qiagen). Genomic DNA was then mixed with Type-it® polymerase, nuclease-free water, and 10 μM reverse and forward primer pairs. Table 1 lists primer sequences and thermal cycling conditions. Melting curves were generated by heating the amplicons from 78C to 88C at a melt ramp rate of 0.1C per second. DNA samples with genotypes that were not clearly determined by high resolution melt analysis were also sent for sequencing. [T1]

Statistical Analysis

A goodness of fit model was used to test whether SNPs followed the Hardy-Weinberg equilibrium. Statistical analysis was first performed using Kaplan-Meier survival curves. Subsequent multivariable analysis was done using Cox regression methods with patient age, gender, race, stage and concomitant carcinoma in situ as covariates. Secondary multivariable analyses were performed,