Genetic Variants in the Wnt/β-Catenin Signaling Pathway as Indicators of Bladder Cancer Risk

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Purpose: Genetic factors that influence bladder cancer risk remain largely unknown. Previous research has suggested that there is a strong genetic component underlying the risk of bladder cancer. The Wnt/β-catenin signaling pathway is a key modulator of cellular proliferation through its regulation of stem cell homeostasis. Furthermore, variants in the Wnt/β-catenin signaling pathway have been implicated in the development of other cancers, leading us to believe that this pathway may have a vital role in bladder cancer development.

Materials and Methods: A total of 230 single nucleotide polymorphisms in 40 genes in the Wnt/β-catenin signaling pathway were genotyped in 803 bladder cancer cases and 803 healthy controls.

Results: A total of 20 single nucleotide polymorphisms were nominally significant for risk. Individuals with 2 variants of *LRP6*: rs10743980 were associated with a decreased risk of bladder cancer in the recessive model in the initial analysis (OR 0.76, 95% CI 0.58−0.99, p = 0.039). This was validated using the bladder genome-wide association study chip (OR 0.51, 95% CI 0.27−1.00, p = 0.049 and for combined analysis p = 0.007).

Conclusions: Together these findings implicate variants in the Wnt/β-catenin stem cell pathway as having a role in bladder cancer etiology.

Key Words: urinary bladder neoplasms; Wnt signaling pathway, polymorphism, single nucleotide; beta catenin; risk

Abbreviations and Acronyms

CSNK1E = casein kinase 1, ε

GWAS = genome-wide association study

LRP = low density lipoprotein receptor related protein

MIBC = muscle invasive bladder cancer

NMIBC = nonMIBC

SNP = single nucleotide polymorphism

Wnt = wingless-type MMTV integration site family

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It was estimated that 54,390 men and 18,300 women would be diagnosed with bladder cancer, and 15,580 men and women would die of this disease in the United States in 2014.[1] Multiple environmental and genetic risk factors have been identified for bladder cancer with smoking and exposure to aromatic amines being the main environmental risk factors.[2] It has also been established that genetics have an important role in the risk of bladder cancer.[3] Recent GWASs identified a total of at least 14 unique genetic loci with a significant effect on bladder cancer risk in populations of European descent.[4−15] However, these risk factors only represent a small portion of the genetic basis of this disease, suggesting that the full spectrum of genetic factors influencing the risk of bladder cancer remains undetermined.

Cancer cells and stem cells have the ability to self-renew. The cancer stem cell hypothesis states that cancer cells hijack the same processes as stem cells for self-renewal and

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proliferation. The Wnt/β-catenin, Notch and Sonic Hedgehog signaling pathways have been associated with stem cell regulation and oncogenesis.[16,17] Specifically the Wnt/β-catenin signaling pathway is a key modulator of cellular proliferation through its regulation of stem cell homeostasis.[18] Signaling through the pathway is complex with multiple layers of regulation. In the absence of binding of Wnt ligands to the cell surface receptor β-catenin is destroyed in a destruction complex made up of APC (adenomatous polyposis coli) and AXIN. This results in β-catenin phosphorylation by CK1 (casein kinase 1) and GSK3 (glycogen synthase kinase 3). Phosphorylation by CK1 and GSK3 causes the ubiquitination and proteasomal degradation of β-catenin. In the normal state Wnt antagonists such as the sFRP (secreted Frizzled-related protein) and DKK (Dickkopf) family members interact with Wnt ligands and prevent pathway activation.[18] If the concentration of Wnt becomes greater than the concentration of sFRP and DKK, Wnt can interact with the FZ (Frizzled) family of receptors and LRP5/6. This activates DSH (disheveled) phosphoprotein, resulting in the degradation of AXIN and an increase in nondegraded β-catenin. The β-catenin concentration in the nucleus increases and activates the transcription of target genes through interactions with TCF (T-cell factor) and LEF (lymphoid enhancer-binding protein).[18]

Recently it was reported that the Wnt/β-catenin pathway is involved in the regulation of stem cells in the bladder epithelium, suggesting that alterations in this vital pathway may result in unrestrained proliferation of bladder epithelium resulting in tumor formation.[19] Therefore, we examined the association between genetic variation in the Wnt/β-catenin pathway genes and the risk of bladder cancer in patients with NMIBC and MIBC using data from an ongoing bladder cancer study at our institution.

MATERIALS AND METHODS

Study Subjects

Bladder cancer cases were recruited from 1999 to 2007 at University of Texas M.D. Anderson Cancer Center and Baylor College of Medicine. They were newly diagnosed, histologically confirmed and previously untreated. Controls were recruited from Kelsey Seybold Clinic, a large multispecialty physician group in Houston, Texas.[20] There were no restrictions on recruitment due to age, gender or stage of bladder cancer. Cases and controls were matched by gender, age $\pm$ 5 years and ethnicity. More than 90% of our patients recruited to the study were white. Therefore, analysis was restricted to this population to limit the confounding effect of population structure. All participants provided written informed consent prior to data and biospecimen collection. The institutional review boards at M.D. Anderson Cancer Center and Baylor College of Medicine approved this study.

Epidemiological and Clinical Data Collection

Demographic and risk factor variables were collected at an interview with each study participant. These variables included age, gender, family history, medical history, occupational exposures and smoking history. Participants were considered never smokers if they had smoked less than 100 cigarettes in a lifetime. Former smokers were those who had quit smoking greater than 1 year after the diagnosis (cases) or the interview (controls). Recent quitters were those who quit smoking greater than 1 month from the date of diagnosis (cases) or interview (controls).

DNA Isolation and Genotyping

Each participant provided a 40 ml peripheral blood sample for genetic and molecular analyses. Laboratory personnel were blinded to case-control status. Genomic DNA was isolated from peripheral blood samples using the QIAamp® DNA Blood Maxi Kit according to the standard protocol. A panel of SNPs related to cancer was developed as previously described.[21] This panel was used to create a custom iSelect® genotyping array that included genes in the Wnt/β-catenin signaling pathway. For each selected gene tagging SNPs ($r^2 \geq 0.8$ and within 10 kb of the gene) and/or potentially functional SNPs (coding SNPs and SNPs in untranslated regions, and promoter and splicing sites) were identified based on the CEU (Utah residents with northern and western European ancestry) HapMap population (http://hapmap.ncbi.nlm.nih.gov/citinghapmap.html.en). Genotyping was performed according to the Infinium® II assay protocol with genotyping calls and quality control assessment done using Illumina® BeadStudio software. The significant SNPs identified from the risk analysis were validated using a GWAS of bladder cancer that was previously done by our research group. Genotyping for this chip was completed at M.D. Anderson Cancer Center and it was done on the Illumina HumanHap610 chip. Detailed methods for this chip were previously described.[5] After removing duplicate samples 352 samples were used.

Statistical Analysis

Stata® 10 statistical software was used to perform most of the statistical analysis. For demographic and clinical variables the Pearson chi-square or Fisher exact test was used to analyze the difference in distribution of categorical variables. The Wilcoxon rank sum test or Student t-test was used to analyze continuous variables. Goodness of fit chi-square analysis was performed to test for Hardy-Weinberg equilibrium in controls. Bladder cancer risk was estimated using the OR and 95% CI, which were calculated using unconditional multivariate logistic regression for the dominant, recessive and additive models of inheritance adjusting for age, gender and smoking status. The false discovery rate (Q value) was calculated to account for the large number of SNPs and tests included in analysis using the Q value package implemented in R (https://www.r-project.org/).

Oncomine™, version 4.4.3 (http://www.oncomine.org), a database of pooled microarray expression data, was queried to search for differential expression data on the