Poster
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UHRF1BP1：A tumor suppressor gene associated with bladder cancer risk in Han Chinese

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BACKGROUND

Seventeen loci have been found to be associated with bladder cancer risk by genome-wide association studies so far in European population. However, little is known about potential contribution of low-frequency and rare variants to bladder cancer susceptibility.

Materials and Methods

We performed a three-stage case-control study including 3,399 bladder cancer patients and 4,647 controls to identify low-frequency and rare variants associated with bladder cancer risk in Han Chinese. We examined exome array data in 1,019 bladder cancer patients and 1,008 controls in the discovery stage using Illumina Human Exome Beadchip. Sequenom MassARRAY and Taqman Probe were used in two replication stages to validate variants identified in discovery stage. Logistic regression was conducted to evaluate single marker association with bladder cancer risk. Gene level-based analysis was applied using SKAT-O method. In vitro experiments were performed to further explore the function of selected gene(s).

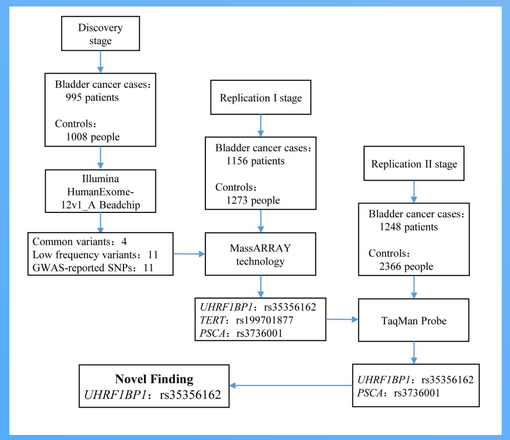

Fig 1. Flow chart of study design and summary of the three-stage study.
In discovery stage, we enrolled 995 bladder cancer cases and 1008 controls to perform exome chip analysis. After filtering, 26 SNVs remained and entered into replication I stage for further validation via MassARRAY technology. In replication I stage, only 3 SNPs survived. These three SNPs entered into replication II stage for validation. Finally, only rs35356162 in *UHRF1BP1* and rs3736001 in *PSCA* survived.

Results

We identified a novel rare coding variant (rs35356162 in UHRF1BP1: G>T, OR=4.332, P=3.62E-07) that increased bladder cancer risk in Han Chinese populations, along with a common variant rs3736001 (G>A, OR=1.265, P=5.00E-06) in the previously reported gene PSCA by GWAS.

Gene-level analysis also showed a significant association of both UHRF1BP1 (P=4.47E-03) and PSCA (P=1.30E-03) with bladder cancer risk.

Additional experiments indicated down-regulation of UHRF1BP1 promoted migration and invasion through epithelial-mesenchymal transition as well as increased proliferation in bladder cancer cell lines.

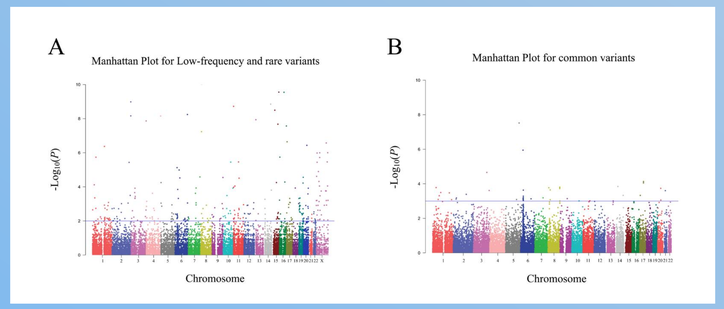

Fig 2. Manhattan plot for exome chip of bladder cancer in Han Chinese populations in the discovery stage.
(A) Manhattan plot for low-frequency and rare variants.
(B) Manhattan plot for common variants.
The X axis represents the chromosomal position and the Y axis represents the $-\log_{10} P$ value. The blue line indicates the filtering threshold of $-\log_{10} P$ value. Blue line in Fig 2 (A) indicated P value was 0.01. Blue line in Fig 2 (A) indicated P value was 0.001.

SNV	Gene	Variant	Locus	Minor/Major Allele	Stage	Genotypes*		MAF		OR (95% CI)	P Value	P Value (SKAT-O test)	Number of SNVs in SKAT-O test
						Cases	Controls	Cases	Controls				
rs35356162	UHRF1BP1	p. Gly152Val	6p21.31	T/G	Discovery	0/21/974	0/3/1000	0.0106	0.0015	7.187 (2.137 – 24.172)	1.44E-03	4.47E-03	13
					Replication I	0/16/1116	0/5/1255	0.0071	0.0020	3.599 (1.314 – 9.855)	1.27E-02		
					Replication II	0/13/1235	0/8/2354	0.0052	0.0017	3.097 (1.280 – 7.493)	1.21E-02		
					Combined	0/50/3325	0/16/4609	0.0074	0.0017	4.332 (2.463 – 7.619)	3.62E-07		
rs3736001	PSCA	p. Glu30Lys	8q24.3	A/G	Discovery	15/216/758	8/171/829	0.1244	0.0928	1.391 (1.137 – 1.703)	1.36E-03	1.30E-03	2
					Replication I	15/241/872	14/228/1018	0.1201	0.1016	1.208 (1.007 – 1.448)	4.15E-02		
					Replication II	18/264/966	17/437/1908	0.1202	0.0997	1.239 (1.060 – 1.447)	6.93E-03		
					Combined	48/721/2596	39/836/3755	0.1214	0.0987	1.265 (1.143 – 1.399)	5.00E-06		

Table 1. Summary of association with bladder cancer risk for rs35356162 and rs3736001 in the three-step stages and gene-based analysis of these two genes.

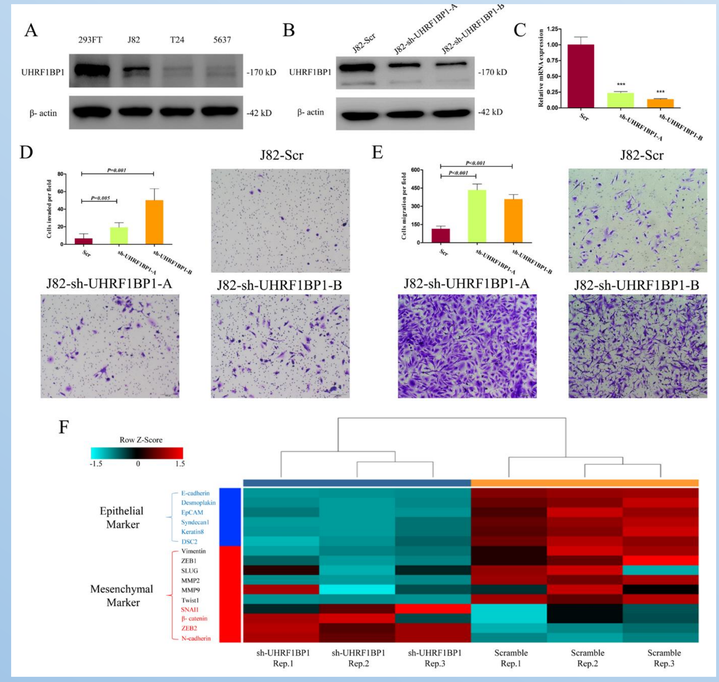

Fig 3. Down-regulation of UHRF1BP1 in J82 cell line can promote cell migration and invasion.
(A) UHRF1BP1 is relatively highly expressed in J82 bladder cancer cell line, β-actin as a loading control. HEK293FT cell line acts as a positive control.
(B) UHRF1BP1 protein expression is significantly inhibited by sh-UHRF1BP1-A&B.
(C) UHRF1BP1 mRNA level is significantly inhibited by sh-UHRF1BP1-A&B.
(D). Representative images of invasion assay for J82 cells infected by scramble and sh-UHRF1BP1-A/B lentiviruses.
(E) Representative images of migration assay for J82 cells infected by scramble and sh-UHRF1BP1-A/B lentiviruses.
(F) Heatmap shows the mRNA expression differences of 16 genes involved in EMT between J82-Scr cells and J82-sh-UHRF1BP1-B cells in triplicates. This PCR array includes six epithelial markers (blue) and ten mesenchymal markers (red).

Conclusion

In conclusion, a rare variant of UHRF1BP1, rs35356162, increases bladder cancer risk in Han Chinese population and UHRF1BP1 might act as a tumor suppressor in bladder cancer development and progression.