Association between interleukin-22 genetic polymorphisms and bladder cancer risk

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OBJECTIVE: The cytokine interleukin-22 (IL-22), which is produced by T cells and natural killer cells, is associated with tumorigenesis and tumor progression in cancers. However, the role of IL-22 in bladder cancer has not been investigated.

MATERIALS AND METHODS: A prospective hospital-based case-control study comprising 210 patients with pathologically proven bladder cancer and 210 age- and gender-matched healthy controls was conducted. The genotypes of 3 common polymorphisms (-429 C/T, +1046 T/A and +1995 A/C) of the IL-22 gene were determined with fluorogenic 5’ exonuclease assays.

RESULTS: Patients with bladder cancer had a significantly higher frequency of the IL-22 -429 TT genotype [odds ratio (OR)=2.04, 95% confidence interval (CI)=1.19, 3.49; p=0.009] and -429 T allele (OR=1.42, 95% CI=1.08, 1.87; p=0.01) than the healthy controls. These findings were still significant after a Bonferroni correction. When stratifying according to the stage of bladder cancer, we found that patients with superficial bladder cancer had a significantly lower frequency of the IL-22 -429 TT genotype (OR=0.48, 95% CI=0.23, 0.98; p=0.04). When stratifying according to the grade and histological type of bladder cancer, we found no statistical association. The IL-22 +1046 T/A and IL-22 +1995 A/C gene polymorphisms were not associated with the risk of bladder cancer.

CONCLUSION: To the authors’ knowledge, this is the first report documenting that the IL-22 -429 C/T gene polymorphism is associated with bladder cancer risk. Additional studies are required to confirm this finding.

KEYWORDS: Interleukin-22; Gene Polymorphism; Bladder Cancer; Case-Control Study.

INTRODUCTION

Bladder cancer is the most common cancer of the urinary tract and is the fourth most commonly diagnosed malignancy in men in the United States. It is estimated that 74,000 new cases of bladder cancer are expected to occur in the United States in 2015 (1). Smoking tobacco and occupational exposure to chemical carcinogens have been established as the strongest risk factors for developing bladder cancer (2-4). It is now commonly accepted that the cause of bladder cancer is the multi-factorial interaction of environmental triggers with genetic susceptibility (5-9). Genome-wide association studies have identified multiple susceptibility loci associated with bladder cancer risk (10-13).

Interleukin-22 (IL-22) is a member of the IL-10 family or IL-10 superfamily, a class of potent mediators of cellular inflammatory responses (14). IL-22 is synthesized by different cell types, including T- and natural killer (NK)-cells and has been reported to mediate crosstalk between inflammatory cells and keratinocytes (15-17). IL-22 is also associated with tumorigenesis and tumor progression in cancers (18,19). The human IL-22 gene is located on the long arm of chromosome 12, on 12q15, approximately 52 and 99 kbp upstream from the IL-26 and interferon loci, respectively and has the same transcriptional orientation as these two adjoining genes (15). Several single nucleotide polymorphisms (SNPs) have previously been identified in the IL-22 gene (20-27).

However, the role of IL-22 in bladder cancer has not been investigated. The aim of this study was to investigate the association between IL-22 gene polymorphisms (-429 C/T, +1046 T/A and +1995 A/C) and the risk of bladder cancer in a Chinese population.
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Table 1 - Primer and probe sequences for the 5'-exonuclease assays of IL-22 polymorphisms.

| SNPs          | genotypes | forward primer | reverse primer | wild-type probe | mutant probe |
|---------------|------------|----------------|----------------|-----------------|--------------|
| -429 C/T      | +1046 T/A  | +1995 A/C      |                |                 |              |
| Forward Primer| AAAATGAGTCCGCTGACAAAAATGC | GCACTATGGAGTCTCCTATCGT | VIC-CTTCTATAGTGACTGAA-NFQ | FAM-CTTCTATAGTGACTGAA-NFQ |
| Reverse Primer| ACACATGGTTTTGCTTATTGAGTCAGAT | CACTAAAGGAGGAGAAGCTGTGT | VIC-CTTCTATAGTGACTGAA-NFQ | FAM-CTTCTATAGTGACTGAA-NFQ |

Table 2 - Distribution of the characteristics of bladder cancer cases and healthy control subjects.

| Characteristic                  | Cases (n=210) | Controls (n=210) | $p$  |
|--------------------------------|---------------|------------------|------|
| Sex (Male/Female)              | 134/76        | 127/83           | 0.48 |
| Age (Years)                    | 61.3 ± 9.8    | 60.7 ± 9.4       | 0.52 |
| Smoking (Ever/Never)           | 111/99        | 85/125           | 0.01 |
| Tumor stage (%)                |               |                  |      |
| Invasive (T2-T4)               | 134(63.8)     | 76(36.2)         |      |
| Superficial (Tis-T1)           |               |                  |      |
| Tumor grade (%)                |               |                  |      |
| High (G2 + G3)                 | 145(69.0)     | 65(31.0)         |      |
| Low (G1)                       |               |                  |      |
| Tumor histological type (%)    |               |                  |      |
| Papillary                      | 157(74.8)     | 53(25.2)         |      |
| Nonpapillary                   |               |                  |      |

Table 3 - Genotype and allele frequencies of IL-22 gene polymorphisms among bladder cancer cases and healthy controls.

| Genotypes | Cases (n=210) | Controls (n=210) | OR (95% CI) | $p$  |
|-----------|---------------|------------------|-------------|------|
| -429 CC   | 68(32.4)      | 79(37.6)         | 1.00(Reference) |      |
| -429 CT   | 84(40.0)      | 98(46.7)         | 1.00(0.64,1.54) | 0.99 |
| -429 TT   | 58(27.6)      | 33(15.7)         | 2.04(1.19,3.49) | 0.009 |
| -429 C allele frequency | 220(52.4) | 256(60.9) | 1.00(Reference) |      |
| -429 T allele frequency | 200(47.6) | 164(39.1) | 1.42(1.08,1.87) | 0.01 |
| +1046 TT  | 90(42.8)      | 79(37.6)         | 1.00(Reference) |      |
| +1046 TA  | 77(36.7)      | 74(35.2)         | 1.12(0.73,1.72) | 0.60 |
| +1046 AA  | 43(20.5)      | 33(15.7)         | 1.19(0.71,2.00) | 0.52 |
| +1046 T allele frequency | 257(61.2) | 268(63.8) | 1.00(Reference) |      |
| +1046 A allele frequency | 163(38.8) | 152(61.2) | 1.12(0.85,1.48) | 0.43 |
| +1995 AA  | 68(32.4)      | 65(30.9)         | 1.00(Reference) |      |
| +1995 AC  | 101(48.1)     | 98(46.7)         | 0.99(0.64,1.53) | 0.95 |
| +1995 CC  | 41(19.5)      | 47(22.4)         | 0.83(0.49,1.43) | 0.51 |
| +1995 A allele frequency | 237(56.4) | 228(54.3) | 1.00(Reference) |      |
| +1995 C allele frequency | 183(43.6) | 192(45.7) | 0.92(0.70,1.20) | 0.53 |

DNA extraction and genotyping

DNA was extracted from peripheral blood lymphocytes using a commercially available Qiagen kit (Qiagen Inc., Valencia, CA, USA). The genotypes of 3 common polymorphisms (-429 C/T, +1046 T/A and +1995 A/C) of the IL-22 gene were determined with fluorogenic 5'-exonuclease assays (TaqMan, Applied Biosystems, Foster City, CA, USA). The primer and probe sequences for the 5'-exonuclease assays of the IL-22 polymorphisms are listed in Table 1. The polymerase chain reaction (PCR) was performed in a Primus 96 plus thermal cycler using a total volume of 5 µl containing 2.5 µl of Universal-MasterMix, 0.125 µl of 40x Assay-by-Design mix, 0.375 µl of H2O and 2 µl of DNA. The reactions were overlaid with 15 µl of mineral oil. The cycling parameters were as follows: 10 min at 94°C for primary denaturation, followed by 40 cycles of 20 s at 92°C and 1 min at 60°C. Fluorescence was measured in a Lambda Fluoro 320 Plus plate reader (MWG Biotech AG, Germany).

Statistical analysis

The data were presented as the means ± standard deviation (SD) or as percentages for categorical variables. Differences between continuous variables were assessed using Student’s t test, while those between categorical variables were evaluated using Pearson’s $x^2$ test. A multivariate logistic regression analysis was used to calculate crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between each IL-22 polymorphism and bladder cancer risk. Statistical significance was obtained from all the participants or their families/surrogates.

SNPs: single nucleotide polymorphisms.
RESULTS

The characteristics of the bladder cancer cases and healthy control subjects are presented in Table 2. A univariate analysis was performed and the results indicated that smoking \( (p=0.01) \) was associated with bladder cancer (Table 2). No significant difference was found between the bladder cancer cases and healthy controls regarding sex or age (Table 2). Regarding the tumor stage of the cases, 134 (63.8%) cases were invasive (T2-T4) bladder cancer and 76 (36.2%) were superficial (Tis-T1) bladder cancer. Regarding the tumor grades of the cases, 65 (31.0%) cases were low grade (G1) bladder cancer and 145 (69.0%) cases were high grade (G2 + G3) bladder cancer. The analysis of the histological types of the cases found that 53 (25.2%) cases were nonpapillary bladder cancer and 157 (74.8%) were papillary bladder cancer.

The genotype frequencies were in agreement with the Hardy-Weinberg equilibrium. The patients with bladder cancer had a significantly higher frequency of the \( IL-22 -429 \) TT genotype (OR=2.04, 95% CI=1.19, 3.49; \( p=0.009 \)) and -429 T allele (OR=1.42, 95% CI=1.08, 1.87; \( p=0.01 \)) than the healthy control subjects (Table 3). The findings remained significant after a Bonferroni correction was implemented. When stratifying according to the stage of bladder cancer, we found that superficial bladder cancer had a significantly lower frequency of the \( IL-22 -429 \) TT genotype (OR=0.48, 95% CI=0.23, 0.98; \( p=0.04 \)) (Table 4). When stratifying according to the grade and histological type of bladder cancer, we found no statistical association (Table 4). The \( IL-22 +1046 \) T/A and \( IL-22 +1995 \) A/C gene polymorphisms were not associated with the risk of bladder cancer (Table 3).

DISCUSSION

In this study, we investigated the association between three common polymorphisms \( (-429 \) C/T, +1046 T/A and +1995 A/C) of the \( IL-22 \) gene and the risk of bladder cancer in a Chinese population. This prospective hospital-based case-control study revealed that the \( IL-22 -429 \) C/T gene polymorphism is associated with bladder cancer risk. To the best of our knowledge, this is the first report in the literature that evaluated the association between \( IL-22 \) gene polymorphisms and the risk of bladder cancer.
bladder cancer. Nine meta-analyses, each of which analyzed between four and twenty-four studies, have provided evidence that the following polymorphisms are associated with increased bladder cancer risk: NQO1 Pro187Ser; PSCA rs2294008 (C>T); XRCC1 Arg399Gln (especially in non-Asian populations); MMP-2 -1306 C/T; MMP-9 -1562 C/T; XPD Lys785Gln; ERCC2 Arg156Arg; Asp312Asn; Lys751Gln; CCND1 G870A (which may modulate the risk of bladder cancer in conjunction with tobacco smoking); XPC A499V (in Caucasian populations); CYP1A1 polymorphisms (especially the 11599G>C, 2455A>G, XPC Arg156Arg; Asp312Asn; Lys751Gln; P272Arg; and A499V (in Caucasian populations); A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); P272Arg; and A499V (in Caucasian populations); B-22 polymorphisms and bladder cancer risk

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REFERENCE

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64(1):9-29, http://dx.doi.org/10.3322/caac.21208.

2. Dowdy D. Tobacco smoking and bladder cancer. JAMA. 2011;306(20): 2216-7; author reply 7.

3. Bachand A, Mundt KA, Mundt DJ, Carlton LE. Meta-analyses of occupa-
tional exposure as a painter and lung and bladder cancer morbidity and mortality 1950-2000. Int J Radiat Oncol Biol Phys. 2010;74(4):101-25, http://dx.doi.org/10.1016/j.ijrobp.2009.03.3326.

4. Mommersen S, Aagaard J. Tobacco as a risk factor in bladder cancer. Car-
cinogenesis. 1985;6(4):335-8, http://dx.doi.org/10.1016/0162-0134(85)90114-0.

5. Wolski R, Genetic polymorphism intermingled with environmental factors substantially contributes to the bladder cancer progression. Cent European J Urol. 2013;66(1):12-3, http://dx.doi.org/10.5177/cej.2013.01.art4k.

6. Volanis D, Kadiytsa T, Galanis A, Delakas D, Logotheti S, Zoumpourlis V. Environmental factors and susceptive polygenic determinant in bladder cancer. Toxicol Lett. 2010;193(1):131-7, http://dx.doi.org/10.1016/j.toxlet.2009.12.018.

7. Honikawa Y, Gu J, Wu X. Genetic susceptibility to bladder cancer with an emphasis on gene-gene and gene-environmental interactions. Curr Opin Urol. 2008;18(5):493-8, http://dx.doi.org/10.1097/MOU.0b013e3283088f8f.

8. Hung RJ, Boffetta P, Brennan P, Malaveille C, Hautefeuille A, Donato F, et al. GST, NAT, SULT1A1, CYPIB1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. Int J Cancer. 2004;109(4):596-604, http://dx.doi.org/10.1002/ijc.20137.

9. Hung RJ, Boffetta P, Brennan P, Malaveille C, Gelatti U, Placiidi D, et al. Genetic polymorphisms and plasma levels of IL-22 contribute to the development of non-small cell lung cancer (38). Recently, a case-control study found that the IL-22 -429 C/T gene polymorphism might be associated with the risk and multifocality of papillary thyroid cancer (26). In 561 colon cancer cases and 722 population controls, an association study suggested that the rs1179251 SNP in IL-22 was associated with the risk of colon cancer (23).

Several limitations to this study should be mentioned. First, these results should be interpreted with caution because the study subjects were Chinese; therefore, the study does not permit extrapolation of the results to different ethnic populations. Second, this is a hospital-based case-control study. Therefore, a selection bias could not be avoided and the subjects may not be representative of the general population. Third, the sample size of our study was relatively small and may not have had adequate statistical power in detecting significant differences. Finally, we did not evaluate the relationships of the 3 common polymorphisms (-429 C/T, +1046 T/A and +1995 A/C) to the plasma levels of IL-22, which may potentially reflect the disease state of patients. Therefore, the association of the IL-22 polymorphisms with plasma levels of IL-22 should be further investigated.

In conclusion, to the authors’ knowledge, this is the first report documenting that the IL-22 -429 C/T gene polymorphism is associated with bladder cancer risk. Additional studies are required to confirm this finding.

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AUTHOR CONTRIBUTIONS

Zhao T and Wu X carried out the molecular genetic studies and drafted the manuscript. Liu J carried out the genotyping. Zhao T and Liu J participated in the design of the study and performed the statistical analysis. Zhao T, Wu X and Liu J conceived the study, participated in its design and coordination and helped to draft the manuscript. All the authors read and approved the final manuscript.

REFERENCES

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64(1):9-29, http://dx.doi.org/10.3322/caac.21208.
and susceptibility to pulmonary tuberculosis. Zhonghua Jie He He Xi Za Zhi. 2012;35(8):596-600.

25. Musolino C, Allegra A, Ferraro M, Aguennouz M, Russo S, Alonci A, et al. Involvement of T2677T multidrug resistance gene polymorphism in Interleukin 22 plasma concentration in B-chronic lymphocytic leukemia patients. Acta Oncol. 2012;51(3):406-8, http://dx.doi.org/10.3109/0284186X.2011.631577.

26. Eun YG, Shin IH, Lee YC, Shin SY, Kim SK, Chung JH, et al. Interleukin 22 polymorphisms and papillary thyroid cancer. J Endocrinol Invest. 2013;36(8):584-7.

27. Suh JS, Cho SH, Chung JH, Moon A, Park YK, Cho BS. A polymorphism of interleukin-22 receptor alpha-1 is associated with the development of childhood IgA nephropathy. J Interferon Cytokine Res. 2013;33(10):571-7, http://dx.doi.org/10.1089/jir.2012.0097.

28. Zhao T et al. IL-22 polymorphisms and bladder cancer risk. CLINICS 2015;70(10):686-690.