Journal of Clinical Laboratory Analysis 20:245–249 (2006)

IL-2 Gene C/T Polymorphism Is Associated With Prostate Cancer

Hsi-Chin Wu,[1] Chao-Hsiang Chang,[1] Lei Wan,[2] Chao-I Wu,[3] Fuu-Jen Tsai,[2,4] and Wen-Chi Chen[1,2]*

[1]*Department of Urology, China Medical University Hospital, Taichung, Taiwan*
[2]*Department of Medical Genetics, China Medical University Hospital, Taichung, Taiwan*
[3]*Department of Plastic Surgery, China Medical University Hospital, Taichung, Taiwan*
[4]*Department of Pediatrics, China Medical University Hospital, Taichung, Taiwan*

Cytokines are reported to be associated with the formation of prostate cancer. Our aim was to investigate whether C/T polymorphisms of the interleukin-2 (IL-2) gene and IL-2 receptor beta (IL-2RB) gene are associated with prostate cancer. We compared the frequency of the polymorphisms of the IL-2 gene and the IL-2RB gene between 96 patients with prostate cancer and 105 healthy male volunteers from the same area (age >60 years). They were followed for at least 5 years. There was a significant difference in distribution of the genotype of the IL-2 gene polymorphism between the prostate cancer group and the control group ($P = 0.017$). The distribution of the TT homozygote of the IL-2 gene was significantly higher in the cancer group (32.3%) than in the control group (16.2%). However, no significant statistical difference was found between the polymorphism of the IL-2 gene and prostate cancer in survival analysis during a 5-year follow up period (log rank test; $P = 0.19$). There was no significant difference in the distribution of the genotype of the IL-2RB gene polymorphism between controls and cancer patients ($P = 0.388$). This study suggests that the IL-2 gene may be associated with susceptibility to prostate cancer in the Taiwan population. J. Clin. Lab. Anal. 20:245–249, 2006. © 2006 Wiley-Liss, Inc.

Key words: interleukin-2 gene (IL-2); interleukin-2 receptor gene (IL-2 R); prostate cancer; single nucleotide polymorphisms (SNPs)

INTRODUCTION

Prostate cancer is the second most common malignancy and the most common in urologic cancer in Taiwanese males. Cytokines are involved in the formation and treatment of prostate cancer (1). Interleukin-2 (IL-2), a cytokine with a potentially antitumor effect has been reported to play a role in prostate cancer. In a study of severe combined immunodeficient mice by Dolman et al. (2). IL-2 effectively inhibited growth and dissemination of lung and bone marrow metastases of human prostate carcinoma. Therefore, the function of IL-2 and/or its receptor may affect the growth of prostate cancer.

IL-2 is an immunoregulatory cytokine. Changes in the IL-2 and IL-2 receptor systems could lead to dysfunctions in the immune system, such as autoimmunity (3–5). A comparative semiquantitative immunohistochemical study by Royuela et al. (6) found that immunoreactions of IL-2 were much higher in prostate cancer samples than in normal prostates. The results suggested that prostate cancer tissue might respond to IL-2 regulation.

While mouse IL-2 is known to be highly polymorphic in the coding sequence that affects the activity of the different strain-specific allotypes (7–9), several human IL-2 polymorphisms have been identified recently, one at position–384 of the promoter region and another, a silent change in the first exon, at position 114 from the initiation codon (10). We also found a T/C polymorphic site of the IL-2 receptor beta gene (IL-2RB; NCBI assay ID: 2048669) located at the 889th nucleotide of mRNA.

*Correspondence to: Wen-Chi Chen, M.D., Ph.D., Department of Urology and Medical Genetics, China Medical University Hospital, No. 2 Yu-Der Road, Taichung 404, Taiwan.
E-mail: drtom@www.cmuh.org.tw

Received 17 June 2005; Accepted 15 September 2005
DOI 10.1002/jcla.20149
Published online in Wiley InterScience (www.interscience.wiley.com).

The polymorphism was identified by restriction enzyme *Hae* III. In this study, we used polymerase chain reaction (PCR)-based restriction analysis to investigate the distribution of IL-2 and IL-2RB gene polymorphisms between the control group and prostate cancer patients. We also analyzed the relationship between clinical parameters of patients with prostate cancer and the distribution of the IL-2 and IL-2RB gene polymorphism genotypes.

MATERIALS AND METHODS

Patient Selection

A total of 96 prostate cancer patients (age 49–96 years; average 70.6 ± 8.97 years) being treated at this hospital were enrolled into this study. The control group consisted of 105 male volunteers over 60 years of age (age 60–87 years; average 66.5 ± 5.08 years) living in the same area. None of them showed signs of prostate cancer by digital rectal examination. Volunteers were tested for serum prostate-specific antigen (PSA) levels (radioimmunoassay), and men with abnormal PSA were either excluded from the normal control group or received further examination, including prostate biopsy, to rule out any prostate disease condition. Specimens from all of the prostate cancer patients were obtained by either transrectal needle biopsy or radical prostatectomy and diagnosed histologically. The PSA levels were measured by radioimmunoassay.

Pathological grading was determined according to the World Health Organization (WHO) criteria and the Gleason pattern. Well, moderately, and poorly differentiated cells generally corresponded to Gleason patterns, 2–4, 5–7, and 8–10, respectively. Clinical and pathological stages were classified by the Tumor-node-metastasis (TNM) system into localized (T1–T2b), advanced (T3–T4), and metastatic (N+ and/or M+) groups (11). There were 29 well differentiated, 30 moderately differentiated, and 37 poorly differentiated prostate cancer tissues according to pathological grading. Patients who received radical prostatectomy were followed and further classified into nadir PSA >4 and <4 groups. A total of 54 patients were treated by hormone therapy after diagnosis of advanced surgically unresectable prostate cancer was made. T1b–T2b patients unfit for curative treatment, such as those who had undergone radical prostatectomy or radiotherapy, as well as symptomatic T3–T4, N+, and/or M+ patients were treated with hormone therapy. Patients were evaluated at 3 and 6 months after initial treatment. Tests included serum PSA measurements, digital rectal examinations, and evaluation of symptoms to assess the treatment response. The response to hormone therapy in prostate cancer patients was defined as serum PSA levels of nadir or below 4 ng/dL within the 6-month follow-up interval. Age of onset of the patients was recorded. Patients were divided into two groups according to their age larger than 70 (>70) or below 70 (≤ 70) years. Informed consent was obtained from individuals in both groups who participated in this study.

Polymerase Chain Reaction

Primers for IL-2 gene G/T polymorphism at position +114 in exon 1 (GENBANK, AF228636) were: 5′-TGGGAAGCACTTAATTATCA-3′ and 5′-TAAC CTCAACTCCTGCCACA-3′. A band product of 262 bp was digested by *Mwo*I (New England Biolabs, Beverly, MA) and separated by electrophoresis on a 12% polyacrylamide gel as described by Matesanz et al. (4). The "G" allele was represented by products with lengths of 151 and 111 bp. The uncut 262 bp product represented the "T" allele.

Primers for the IL-2RB gene C/T polymorphism were: 5′-AAGGACACCATTCCGTGGCT-3′ and 5′-CCGG TGTTCCTGCAGTTGAT-3′. PCR amplification yielded a band of 101 bp. After digestion with *Hae-III* (New England Biolabs), the 63- and 38-bp fragments were separated on 3% agarose gel by electrophoresis, stained with ethidium bromide, and visualized with ultraviolet light. The resulting products were classified as digestible (CC homozygote), indigestible (TT homozygote), and combined C/T heterozygote.

Polymerase chain reactions (PCRs) were carried out to a total volume of 50 μL, containing genomic DNA 10–20 ng, 2 μL (2–6 pmol) of each primer, 1 × Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer Foster City, CA). The cycling condition for the IL-2 gene was set as follows: one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 30 sec, 57 °C for 30 sec, and 72 °C for 30 sec, and one final cycle of extension at 72 °C for 7 min. Cycling conditions for the IL-2RB gene were the same as those for IL-2 except that the annealing polymerization temperature was decreased from 62 °C to 52 °C at a rate of 0.5 °C in every cycle.

All cancer patients were followed and survival status was recorded for at least 5 years after the initial diagnosis of prostate cancer. Survival rate was calculated by Kaplan-Meier survival analysis with log-rank test according to the time of survival. The software used for the calculation is the Statistical Package for the Social Science system (SPSS, Chicago, IL). Results were considered statistically significantly when the probability of findings occurring by chance was less than 5% ($P < 0.05$).

RESULTS

The genotype distributions for the IL-2 gene obtained from the patients and controls are shown in Table 1. There were 21 prostate cancer patients with the GG homozygote genotype, 31 with the TT homozygote genotype, and 44 with the TG heterozygote genotype of the IL-2 gene. There were significant differences in genotype distribution of the IL-2 gene between patients and controls (Table 1; $P = 0.017$). The distribution of the IL-2 gene TT genotype in prostate cancer patients (31 patients; 32.3%) was higher than in the controls (17 patients; 16.2%). During the 5-year follow-up period, there were three deaths among prostate cancer patients with genotype of GG, seven deaths among those with TT homozygote genotype, and 17 deaths among those with TG heterozygote genotype of the IL-2 gene. The Kaplan-Meier survival curve for the IL-2 gene C/T polymorphism and IL-2RB gene T/G polymorphism for patients with prostate cancer is shown in Fig. 1. There was no statistical significance among the three genotypes in 5-year survival time in patients with prostate cancer (long-rank test; $P = 0.310$ for IL-2 and $P = 0.915$ for IL-2RB).

The distribution of the IL-2 gene C/T polymorphism did not differ significantly among these three groups ($P = 0.936$). There were 53 localized, 20 advanced, and 23 metastasis stages according to clinical staging, none of which were statistically significant ($P = 0.552$). Patients with prostate cancer who received hormonal therapy were classified into response (42 patients) and nonresponse (12 patients) groups. There was no significant difference in the distribution of the IL-2 gene G/T polymorphism ($P = 0.36$) between them. There was no significant difference in the distribution of the IL-2 C/T polymorphism between the two age groups ($P = 0.55$) (Table 2).

There was no statistical significance in the genotype distribution of the IL-2RB gene between the patients and controls (Table 1; $P = 0.388$).

DISCUSSION

Few reports regarding the association between the IL-2 gene polymorphism and prostate cancer have been published. In this study, we demonstrated an association between the IL-2 genotype and prostate cancer. The distributions of genotypes at position+114 in exon 1 differed significantly between patients and controls. The frequency of the TT homozygote was higher in patients with prostate cancer than in the controls. However, no association was observed between IL-2 gene polymorphisms and clinical parameters. Furthermore, the distribution of the IL-2RB gene C/T polymorphism did not differ significantly between prostate cancer patients and controls. Therefore, the IL-2 gene might be associated with tumor formation but not tumor progression.

The IL-2 gene is located on chromosome 4q26. The relationship between prostate cancer progression and the IL-2 gene has been shown in several reports. Using an animal model, Moody et al. (12) found that high local concentrations of IL-2 stimulated the elimination of large local burdens of prostate cancer. This elimination resulted in a weak, but detectable systemic immune response against wild-type prostate cancer cells. Hrouda and Dalgleish (13) were the first to report that prostate cancer could potentially be treated with IL-2 gene therapy. Since then, several phase I and II of IL-2 gene therapy studies have been conducted in patients with

TABLE 2. Distribution of the interleukin-2 gene exon 1, position +114 G/T polymorphism in prostate cancer patients according to patient age, clinical staging, pathological grading, and response to hormone therapy (Fisher's exact test)

Genotype	TT	TG	GG	Total	*P*-value
Grading					0.936
Well differentiated	11	12	6	29	
Moderately differentiated	9	15	6	30	
Poorly differentiated	11	17	9	37	
Staging					0.552
Local	20	24	9	53	
Advanced	6	8	6	20	
Metastasis	5	12	6	23	
Age					0.55
70	13	22	12	50	
>70	18	22	9	46	
Responsiveness					0.36
Response	9	22	11	42	
No-response	5	5	2	12	

TABLE 1. Distribution of the interleukin-2 (IL-2) gene exon 1, position +114 G/T polymorphism and the IL-2 receptor beta (IL-2RB) gene C/T polymorphism between the healthy control subjects and the prostate cancer patients by chi-squared test

IL-2	TT	TG	GG	Total	*P* value
Control	17 (16.2%)	66 (62.9%)	22 (20.9%)	105 (100.0%)	0.017
Cancer patient	31 (32.3%)	44 (45.8%)	21 (21.9%)	96 (100.0%)	
IL-2RB	TT	TC	CC	Total	*P* value
Control	41 (39.0%)	35 (33.3%)	29 (27.7%)	105 (100.0%)	0.388
Cancer patient	29 (30.2%)	39 (40.6%)	28 (29.2%)	96 (100.0%)	

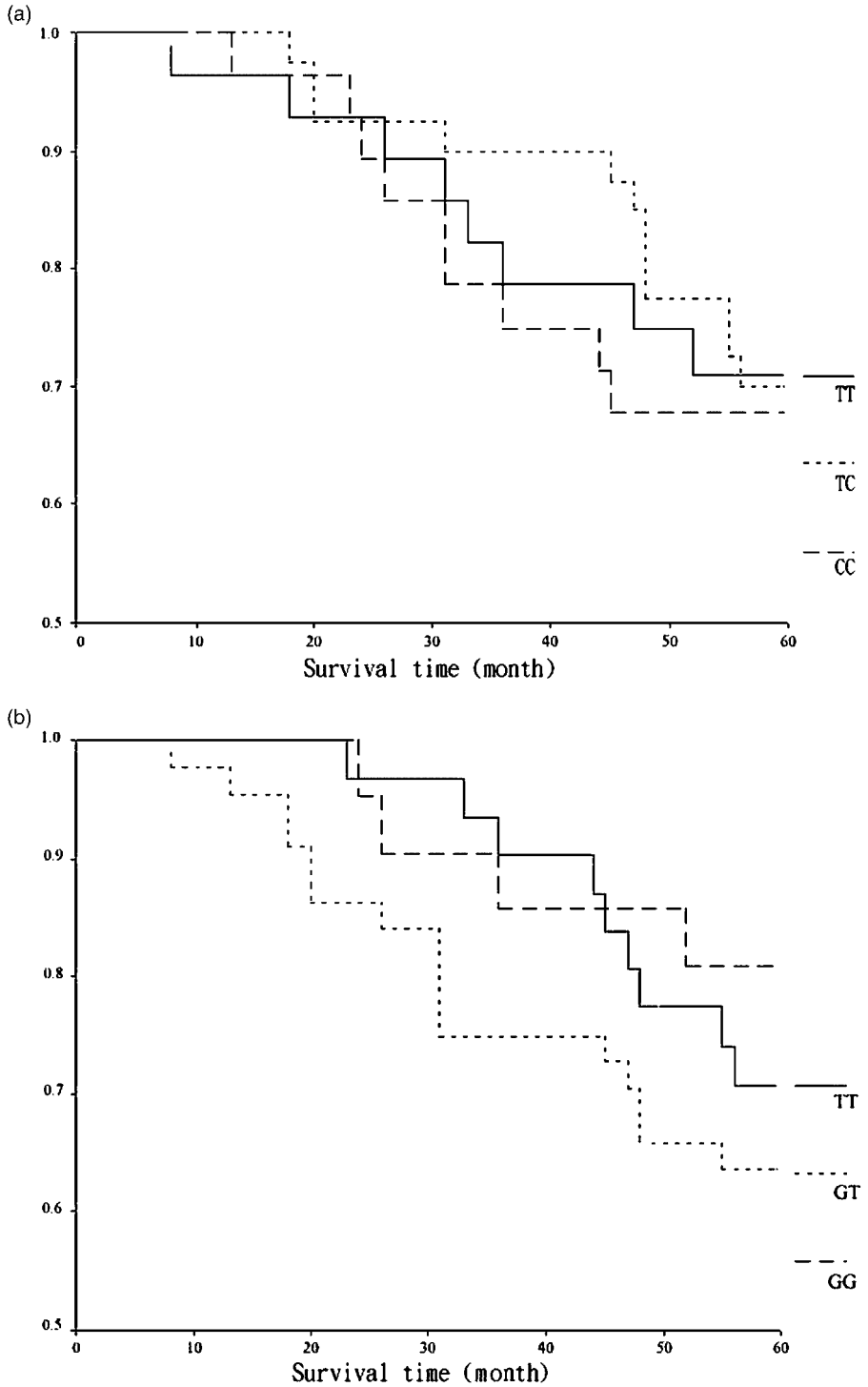

Fig. 1. Kaplan-Meier survival curve analysis of the C/T polymorphism of the IL-2 gene in patients with prostate cancer (**a:** log rank test; $P = 0.310$) and the G/T polymorphism of IL-2 receptor beta gene (**b:** log rank test; $P = 0.915$).

prostate cancer. For example, Belldegrun et al. (14) conducted the first phase I clinical trial of IL-2 gene therapy and demonstrated and transient lowering of PSA to the baseline level after therapy. Triest et al. (15) investigated the effect of systemic IL-2 treatment on metastatic prostate carcinoma using a xenograft tumor model and found that systemic IL-2 therapy can induce an antitumor response in prostate tumors and control their growth and metastasis. Hautmann et al. (16) also reported an effective and nontoxic effect of IL-2

treatment in the local intratumor immunotherapy for prostate cancer.

Although Royuela et al. (6) found higher levels of IL-2 and IL-2RB in prostate cancer tissue than in the normal prostate indicating a positive relationship between prostate cancer and IL-2RB. Other studies have not shown clear evidence of the relationship between prostate cancer and IL-2RB. For example, Lloyd et al. (17) failed to find a positive relationship between IL-2RB expression and the degree of cell-mediated immune response to the tumor. Our results may confirm their findings.

There are some limitations of this study. For example, our patient sample size was small. Our data might be of limited value and therefore an additional study of more cases is needed. We have been unable to find a significant association between IL-2RB C/T polymorphism and prostate cancer. However, the possibility of a type II error must be kept in mind in view of the small sample size.

There was no significant difference in survival time among genotypes of the IL-2 gene C/T or the IL-2RB gene G/T polymorphism in patients with prostate cancer. A further study spanning more than 10 years of survival time is warranted. In conclusion, the IL-2 gene C/T polymorphism is associated with prostate cancer and therefore could be a possible genetic marker for studying the tumorigenesis of prostate cancer.

REFERENCES

1. Harris DT, Matyas GR, Gomella LG, et al. Immunologic approaches to the treatment of prostate cancer. Semin Oncol 1999;26:439–447.
2. Dolman CS, Mueller BM, Lode HN, Xiang R, Gillies SD, Reisfeld RA. Suppression of human prostate carcinoma metastases in severe combined immunodeficient mice by interleukin 2 immunocytokine therapy. Clin Cancer Res 1998;4:2551–2257.
3. Horak I. Immunodeficiency in IL-2-knockout mice. Clin Immunol Immunopathol 1995;76:S172–S173.
4. Matesanz F, Fedetz M, Collado-Romero M, et al. Allelic expression and interleukin-2 polymorphisms in multiple sclerosis. J Neuroimmunol 2001;119:101–105.
5. Sadlack B, Lohler J, Schorle H, et al. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+T cells. Eur J Immunol 1995;25:3053–3059.
6. Royuela M, De Miguel MP, Bethencourt FR, Fraile B, Arenas MI, Paniagua R. IL-2, its receptors, and bcl-2 and bax genes in normal, hyperplastic and carcinomatous human prostates: immunohistochemical comparative analysis. Growth Factors 2000;18:135–146.
7. Matesanz F, Alcina A, Pellicer A. A new cDNA sequence for the murine interleukin-2 gene. Biochim Biophys Acta 1992;1132: 335–336.
8. Matesanz F, Alcina A, Pellicer A. Existence of at least five interleukin-2 molecules in different mouse strains. Immunogenetics 1993;38:300–303.
9. Matesanz F, Alcina A. Induction of autoantibodies to different interleukin-2 allotypes. J Autoimmun 1999;12:221–227.
10. Matesanzl F, Delgado C, Fresno M, Alcinal A. Allelic selection of human IL-2 gene. Eur J Immunol 2000;30:3516–3521.
11. Aus G, Abbou CC, Pacik D, et al. EAU guidelines on prostate cancer. Eur Urol 2001;40:97–101.
12. Moody DB, Robinson JC, Ewing CM, Lazenby AJ, Isaacs WB. Interleukin-2 transfected prostate cancer cells generate a local antitumor effect in vivo. Prostate 1994;24:244–251.
13. Hrouda D, Dalgleish AG. Gene therapy for prostate cancer. Gene Ther 1996;3:845–852.
14. Belldegrun A, Tso CL, Zisman A, et al. Interleukin 2 gene therapy for prostate cancer: phase I clinical trial and basic biology. Hum Gene Ther 2001;12:883–892.
15. Triest JA, Grignon DJ, Cher ML, et al. Systemic interleukin 2 therapy for human prostate tumors in a nude mouse model. Clin Cancer Res 1998;4:2009–2014.
16. Hautmann SH, Huland E, Huland H. Local intratumor immunotherapy of prostate cancer with interleukin-2 reduces tumor growth. Anticancer Res 1999;19:2661–2663.
17. Lloyd SN, McClinton S, Brown IL, et al. Epidermal growth factor receptor, nuclear proliferation antigen and interleukin-2 receptor expression in prostatic cancer. J Clin Lab Immunol 1990;32:55–58.