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ORIGINAL ARTICLE

Clinical study of IL-18 and NANOG gene polymorphisms in prostate cancer patients*

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Abstract

Objective Recent studies have shown abnormal expression of NANOG and IL-18 to be related to cancer. However, the molecular mechanism by which IL-18 and NANOG gene polymorphisms are associated with prostate cancer is unclear. In this study, we examined whether IL-18 and *NANOG* gene polymorphisms and their interaction with prostate cancer-related risk factor are associated with the susceptibility to and clinicopathological development of prostate cancer among Chinese men.

Methods Polymorphisms in the *NANOG* and IL-18 genes were evaluated for susceptibility in 120 patients with prostate cancer. The control group consisted of 125 samples from Chinese men. Genotyping was conducted using TaqMan allelic discrimination assays. Statistical analysis was performed using SPSS software.

Results No association of *NANOG* and IL-18 gene polymorphisms and overall prostate cancer susceptibility was detected. The IL-18-607 CC genotype was significantly associated with a higher tumor grade (P = 0.025) and stage (P =0.001). The IL-18-137 GG genotype correlated with a higher tumor grade (P = 0.028) and stage (P = 0.008). The IL-18-137G allele was significantly more frequent in patients with lymph node metastasis (P = 0.035). The IL-18-607 CC genotype was associated with distant metastasis (P = 0.025). However, no significant association was observed between *NANOG* polymorphisms and any clinicopathological feature. The Cox proportional hazard model showed that tumor grade and stage grouping were independent prognostic factors in IL-18, while IL-18 polymorphism was not. Polymorphism variants in the IL-18 (IL-18-607 and IL-18-137) and *NANOG* (genotypes AC) genes might be associated with a worse prognosis of patients with prostate cancer.

Conclusion *NANOG* may be associated with the early stages of prostate cancer carcinogenesis. IL-18 and NANOG gene polymorphisms may play a major role in the growth, invasion, and metastasis of prostate cancer.

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Embryonic stem cells are known as "cells that start the tumor" [1]. These cells possess self-renewal capacity, which enables differentiation into heterogeneous mature cells types, including tumor cells. Numerous primary non malignant and malignant tumor derived human prostate epithelial cell lines have been developed using a retroviral vector encoding the human telomerase reverse transcriptase. These cell lines exhibit characteristics of stem cells and express embryonic stem (ES) cell markers, such as *NANOG*, octamer 4 (*OCT4*), and SRY box 2(*Sox2*) [2]. The NANOG gene is located on chromosome 12 and plays a vital role in cell differentiation. Recent studies found that abnormal expression of NANOG, OCT4, and SOX2 is related to colorectal and lung cancer [3–7].

Although these factors are necessary for stem cells to acquire pluripotency, they have also been suggested to possess oncogenic potential in normal cells. However, the role of these factors in normal cells and cancer cells

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is unclear. Additional studies are needed to determine the influence of these factors on the proliferation and metastatic potential of cancer.

Recent studies showed that prostate tumor cells secrete interleukin (IL)-18 in response to interferon-γ in the tumor microenvironment and that IL-18 acts as an autocrine or paracrine factor in the tumor [8]. In some animal models, IL-18 gene transfection into tumor cells enhanced both specific and nonspecific antitumor immune responses, indicating that if the IL-18 gene is transferred into dendritic cells, it can induce highly effective antitumor immune responses [9]. These findings suggest an association between the susceptibility to cancer and IL-18 gene. We previously showed that IL-18 plays an important role in prostate cancer growth and metastasis. Additionally, our research and a recent study revealed a correlation between serum IL-18 and vascular endothelial growth factor (VEGF) levels in patients with prostate and ovarian cancer [10–11]. The IL-18 gene is located on chromosome 11q22. Two functional gene polymorphisms, -607A/C and -137G/C, are found in its promoter region. Giedraitis *et al.* analyzed the IL-18 gene promoter sequence and found a change from the C allele to A allele at position -607 and change from G to C at position-137 [12]. Our study showed that polymorphisms in the IL-18 promoter affect prostate cancer progression and prognosis. IL-18 is strongly correlated with a higher tumor grade and stage, lymph node involvement, and distant metastasis [13]. However, the molecular mechanism by which IL-18 gene polymorphisms are associated with prostate cancer is unclear.

Therefore, in this study, we recruited 245 participants, including 120 patients with prostate cancer and 125 healthy men, to determine whether IL-18 and *NANOG* gene polymorphisms and their interactions with prostate cancer-related risk factors are associated with the susceptibility to and clinicopathological development of prostate cancer among the Chinese men.

Materials and methods

Patients

A total of 120 patients with prostate cancer who underwent radical prostatectomy between 2005 and 2011 in the Department of Urology, The Affiliated Hospital of Nantong University (China) were investigated. To eliminate the influence of other diseases, we excluded patients with infectious diseases and diabetes mellitus. No patient with prostate cancer was subjected to chemotherapy, hormonal therapy, or radiotherapy before surgery. The patients including non-metastases in 80 cases and metastases in 40 cases were aged 58–85 years (mean age, 70.43 ± 11.14 years). The tumor stage was assigned according to the Whitmore-Jewett stage. The tumor was assigned according to the Gleason score. Patients were divided into groups with low (≤ 6) and high (> 6) Gleason scores. The patients and tumor characteristics are listed in Table 1. Bone metastases were assessed by bone X-ray and bone scanning. Extraosseous metastases were assessed by surgical biopsy. Recurrence was defined as a significant elevation in PSA and/or new symptoms because of local tumor recurrence. The control group contained 125 healthy volunteers (mean age 70.70 ± 9.41 years) who visited the general health check-up division at The Affiliated Hospital of Nantong University. Selection criteria for controls were no evidence of any personal or family history of cancer or other serious illness. The mean age of the control group was 69.4 years. The median follow-up time was 16 months (range: 6–38 months) after surgery, and the study was performed with the approval of the ethics committee of the Chinese Human Genome.

Table 1 Clinicopathological characteristics of prostate cancer patients (*n*)

Characteristic	No. of patients	%
Age (years)		
Mean	70.43 ± 11.14	
Range	58–85	
Tumor stage		
Lymph node metastasis		
A	5	4.2
B	67	55.8
C	10	8.3
D	38	31.7
Lymph node metastasis		
Negative	70	58.3
Positive	50	41.7
Metastasis		
Negative	80	66.7
Positive	40	33.3
Grade		
≤ 6	63	52.5
> 6	57	47.5

DNA extraction

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes by the salting-out method. Briefly, 5 mL of blood was mixed with Triton lysis buffer (0.32 M sucrose, 1% Triton_100, 5 mM $MgCl_2$, H_2O, and 10 mM Tris-HCl, pH 7.5). Leucocytes were centrifuged and washed with H_2O. The pellet was incubated with proteinase K at 56°C and subsequently salted out at 48°C using a saturated NaCl solution. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was dissolved in 300 mL H_2O.

IL-18 genotype

Genotyping of the two IL-18 polymorphisms was performed using predesigned TaqMan single-nucleotide polymorphism (SNP) Genotyping Assays (Applied Biosystems, USA). The Assays-on-Demand SNP genotyping kit was used for polymerase chain reaction (Applied Biosystems). Single-nucleotide polymorphism amplification assays were performed according to the manufacturer's instructions. Briefly, a 25-μL reaction solution containing 10 ng of DNA was mixed with 12.5 μL of 2 × TaqMan Universal PCR Mix (Applied Biosystems) and 1.25 μL of predeveloped assay reagent from the SNP genotyping product (Applied Biosystems) containing two primers and two MGB TaqMan probes. Reaction conditions consisted of preincubation at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Amplifications and analysis were performed in an ABI Prism 7500 Sequence Detection System (Applied Biosystems) using SDS 1.4 software for allelic discrimination (Applied Biosystems). The following SNPs were typed: IL-18-137 G/C (rs187238) and IL-18-607 A/C (rs1946518).

NANOG genotype

Tag SNPs were selected using Haploview software 4.2 (Mark Daly's laboratory of Broad Institute, USA) [14] based on the GIH population data of HapMap (HapMap Data Rel 27 Phase II + III, Feb 09, on NCBI B36 assembly, dbSNP b126). Tag SNPs that captured all known common SNPs (with minor allele frequencies of > 0.1) in *NANOG*, with a pairwise correlation $r^2 > 0.8$, were selected. Tagger SNP rs1105786 (minor allele frequency, MAF =0.376) was found to represent known SNPs in haplotype blocks 2 in *NANOG* in the Chinese population.

Statistical analysis

SNP allele frequencies were tested for departure from Hardy-Weinberg equilibrium before analysis. Genotype frequencies were compared using the Pearson χ^2 test for 2 × 2 tables or Fisher's exact test when the expected frequency value was < 5. Patients were classified in a dichotomous manner for each of the following clinical parameters: tumor diameter, nuclear grade, tumor stage, lymph node metastasis, distant metastasis, stage grouping, and survival. The distribution of polymorphisms for each parameter was studied by analyzing the genotype group and allele frequency. Odds ratios (ORs) and significance (*P* values) were also calculated. The influence of each variable on survival was assessed using the Cox proportional hazard model. Values of $P < 0.05$ were considered significant. SPSS software version 11.5 was used for all statistical analyses (SPSS, Inc., USA).

Table 2 Associations of IL-18 genotypes with tumor risk (*n*, %)

IL-18 Polymorphisms	PC patients	Healthy controls	OR (95% CI)	*P*
-137 C/G Genotype				
CC	6 (5.0)	10 (8.0)	1.55 (0.45–4.05)	0.522
CG	47 (39.2)	40 (32.0)	1.00 (Reference)	
GG	67 (55.8)	75 (60.0)	0.78 (0.47–1.15)	0.725
Allele				
C	56 (23.3)	75 (30.0)	1.00 (Reference)	
G	184 (76.7)	175 (70.0)	1.45 (0.75–1.87)	0.072
-607 A/C Genotype				
AA	13 (10.8)	10 (8.0)	1.22 (0.56–1.96)	0.657
AC	61 (50.8)	65 (52.0)	1.00 (Reference)	
CC	46 (38.3)	50 (40.0)	0.787 (0.61–1.33)	0.322
Allele				
A	114 (47.5)	110 (44.0)	1.00 (Reference)	
C	126 (52.5)	115 (46.0)	1.36 (0.81–1.69)	0.381

Results

Correlation of IL-18 gene polymorphisms with prostate cancer clinicopathological characteristics

This case-control study revealed similar frequencies in the distribution of IL-18-137 and -607 polymorphisms between healthy controls and patients with prostate cancer. Table 2 presents the genotype distributions and statistical analysis results. The observed genotype frequencies were in accordance with Hardy-Weinberg equilibrium. The association of the IL-18 genotypes with tumor grade and stage were shown in Table 3. Genotype GG of IL-18-137 was associated with a more advanced cancer stage (OR: 2.61; 95% CI: 1.15–5.37; $P = 0.008$) and higher tumor grade (OR: 3.32; 95% CI: 1.16-8.17; $P = 0.028$). The IL-18-137 G allele was correlated with a more advanced stage (OR: 1.73; 95% CI: 1.04–3.42; $P = 0.027$) and higher tumor grade (OR: 2.13; 95% CI: 0.98–4.12; $P = 0.040$). The IL-18-607 CC genotype was significantly more frequent in patients with more advanced cancer stages (OR: 3.82; 95% CI: 1.67–7.67; $P = 0.001$) and higher tumor grade (OR: 3.11; 95% CI: 1.05–10.25; $P = 0.025$). The IL-18-607 C allele was associated with a more advanced cancer stage (OR: 2.37; 95% CI: 1.28–3.73; $P = 0.001$). The associations of IL-18 genotypes with lymph node metastasis and distant metastasis were shown in Table 4. The IL-18-137G allele was significantly more frequent in patients with lymph node metastasis (OR: 3.82; 95% CI:0.95–15.17; $P = 0.035$). The IL-18-607 CC genotype was associated with distant metastasis (OR: 2.71; 95% CI: 1.25–6.14; $P = 0.025$).

Table 3 Associations of IL-18 genotypes with tumor stage, and grade (*n*, %)

IL-18 polymorphisms	Tumor stage		Odds ratio (95% CI)	*P*	Tumor grade Odds		Ratio (95% CI)	*P*
	A–B	C–D			≤ 6	> 6		
-137 C/G Genotype								
CC	4 (5.6)	2 (4.2)	1.25 (0.21–7.81)	0.625	3 (4.7)	3 (5.3)	1.36 (0.15–16.27)	0.620
CG	35 (48.6)	14 (29.2)	1.00 (Reference)		35 (55.6)	11 (193)	1.00 (Reference)	
GG	33 (45.8)	32 (66.7)	2.61 (1.15–5.37)	0.008	25 (39.7)	43 (75.4)	3.32 (1.16–8.17)	0.028
Allele								
C	35 (29.2)	32 (24.8)	1.00 (Reference)		55 (42.3)	32 (26.2)	1.00 (Reference)	
G	85 (70.8)	97 (75.2)	1.73 (1.04–3.42)	0.027	75 (57.7)	90 (73.7)	2.13 (0.98–4.12)	0.040
-607 A/C Genotype								
AA	8 (11.1)	3 (6.2)	0.83 (0.26–2.37)	0.617	5 (7.9)	4 (7.0)	1.17 (0.27–5.05)	0.782
AC	44 (61.1)	21 (43.8)	1.00 (Reference)		44 (69.8)	29 (50.9)	1.00 (Reference)	
CC	20 (27.8)	24 (50.0)	3.82 (1.67–7.67)	0.001	14 (22.2)	24 (42.1)	3.11 (1.05–10.25)	0.025
Allele								
A	57 (43.2)	45 (35.4)	1.00 (Reference)		35 (26.9)	23 (17.7)	1.00 (Reference)	
C	75 (56.8)	82 (64.6)	2.37 (1.28–3.73)	0.001	95 (73.1)	107 (82.3)	1.78 (0.87–4.52)	0.153

Table 4 Associations of IL-18 genotypes with Lymph node metastasis, metastasis and Stage grouping (*n*, %)

IL-18 polymorphisms	Lymph nodemetastasis		OR (95% CI)	*P*	Metastasis		OR (95% CI)	*P*
	A–B	C–D			≤ 6	> 6		
-137 C/G Genotype								
CC	2 (2.9)	1 (2.0)	0.84 (0.88–1.12)	0.672	2 (2.5)	1 (2.5)	2.81 (0.32–13.27)	0.427
CG	30 (42.9)	23 (46.0)	1.00 (Reference)		35 (43.8)	7 (17.5)	1.00 (Reference)	
GG	38 (54.2)	26 (52.0)	1.79 (0.33–8.35)	0.436	43 (53.7)	32 (80.01)	1.98 (0.92–5.17)	0.163
Allele								
C	65 (43.3)	5 (16.7)	1.00 (Reference)		42 (25.1)	11 (16.2)	1.00 (Reference)	
G	85 (56.7)	25 (83.3)	3.82 (0.95–15.17)	0.035	125 (74.9)	57 (83.8)	1.57 (0.82–3.50)	0.317
-607 A/C Genotype								
AA	3 (4.3)	2 (4.0)	0.87 (0.89–1.03)	0.343	5 (6.3)	2 (5.0)	2.47 (0.67–7.15)	0.168
AC	40 (57.1)	16 (32.0)	1.00 (Reference)		47 (58.7)	15 (37.5)	1.00 (Reference)	
CC	27 (38.6)	32 (64.0)	2.62 (0.68–9.67)	0.152	28 (35.0)	23 (57.5)	2.71 (1.25–6.14)	0.025
Allele								
A	60 (46.2)	5 (18.5)	1.00 (Reference)		61 (34.7)	21 (28.8)	1.00 (Reference)	
C	70 (53.8)	22 (81.5)	2.98 (0.89–8.93)	0.057	115 (65.3)	52 (71.2)	1.45 (0.83–3.45)	0.237

Table 5 Associations of Nanog genotypes with tumor risk (*n*, %)

Nanog polymorphisms	PC patients	Healthy controls	OR (95% CI)	*P*
Genotype				
TT	14 (11.7)	10 (8.0)	1.31 (0.64–1.75)	0.658
CT	60 (50.0)	65 (52.0)	1.00 (Reference)	
CC	46 (38.3)	50 (40.0)	0.897 (0.75–1.52)	0.402
Allele				
T	120 (47.6)	110 (44.0)	1.00 (Reference)	
C	132 (52.4)	115 (46.0)	1.16 (0.87–1.59)	0.301

Correlation of NANOG gene polymorphisms with prostate cancer clinicopathological characteristics

The observed genotype frequencies of the *NANOG* gene polymorphisms studied in healthy controls were in accordance with Hardy–Weinberg equilibrium (Table 5). No significant differences were observed in the frequency distribution of *NANOG* rs11055786 polymorphisms between patients with prostate cancer and healthy controls, both at the genotypic and allelic levels (Table 5). We performed binary logistic regression analysis to evaluate the correlations between *NANOG*

Table 6 Correlation of Nanog gene polymorphisms with prostate cancer clinicopathological characteristics

Nanog	TT	CT + CC	OR (95%CI)	*P*	T	C	OR (95%CI)	*P*
Clincal stage								
A + B	41 (64.1)	31 (55.4)	1 (Reference)		67 (55.8)	79 (65.8)	1 (Reference)	
C + D	23 (35.9)	25 (44.6)	1.70 (0.82–3.52)	0.28	53 (44.2)	41 (34.2)	1.08 (0.72–2.15)	0.65
Grade								
≤ 6	35 (59.3)	38 (53.5)	1 (Reference)		62 (51.7)	67 (55.8)	1 (Reference)	
> 6	24 (40.7)	33 (46.5)	0.82 (0.40–1.72)	0.72	58 (48.3)	53 (44.2)	0.65 (0.49–1.24)	0.37
Lymphy node								
Negative	24 (61.5)	46 (56.8)	1 (Reference)		65 (54.2)	70 (58.3)	1 (Reference)	
Positive	15 (38.5)	35 (43.2)	6.08 (1.82–20.12)	0.24	55 (45.8)	50 (41.7)	1.26 (0.65–2.27)	0.34
Metastasis								
Negative	38 (67.9)	42 (65.6)	1 (Reference)		83 (69.2)	75 (62.5)	1 (Reference)	
Positive	18 (32.1)	22 (34.4)	1.81 (0.86–3.72)	0.39	37 (30.8)	45 (37.5)	0.56 (0.35–1.21)	0.18

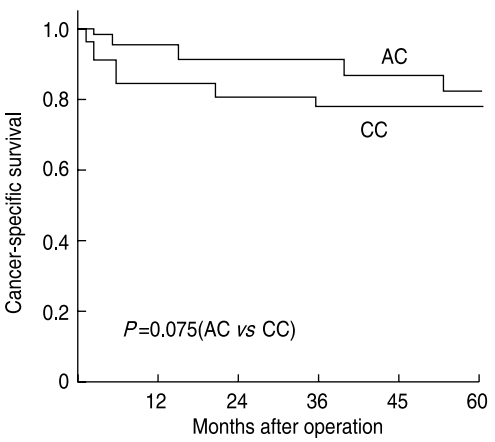

Fig. 1 Kaplan-Meier overall survival estimate according to IL-18-607 polymorphism. Differences between curves were evaluated by the Log-rank test.

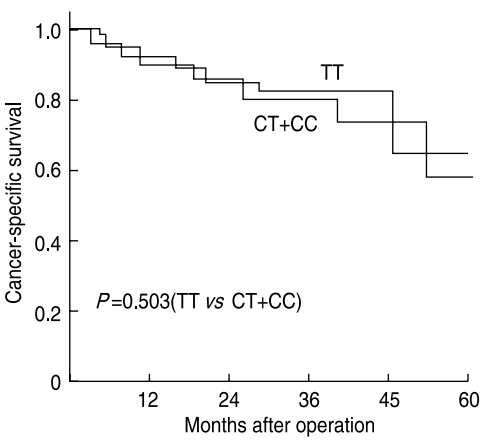

Fig. 2 Kaplan-Meier overall survival estimate according to Nanog polymorphism. Differences between curves were evaluated by the Log-rank test.

gene polymorphisms and clinicopathological features in 120 patients. However, no significant association between the *NANOG* rs1105786 polymorphism and any clinicopathological features was detected (Table 6).

IL-18 and Nanog polymorphisms in cancer survival

Thirty-four patients died of cancer-related causes during the follow-up period. Kaplan-Meier curves were calculated for cancer-specific survival by IL-18-607 genotype (AC and CC) and Nanog genotype (TT and CT + CC) (Fig. 1 and Fig. 2). Patients with the IL-18-607 genotype AC genotype showed more favorable cancer-specific survival than those with the CC genotype (P = 0.076; Log-rank test). However, the NANOG genotypes TT and CT + CC were not significantly correlated with cancer survival (P = 0.503; Log-rank test). The Cox proportional hazard model revealed that tumor grade and stage grouping were independent prognosis factors (Table 7). However, IL-18 polymorphisms, at least in this series of patients, did not serve as independent prognosis factors.

Discussion

CSCs are important in carcinogenesis and treatment resistance and may lead to metastasis. Pluripotency associated transcription factors, including *NANOG*, *Sox2*, and *OCT4*, are known to regulate cellular identity in embryonic stem cells [15] and were recently identified in epithelial malignancies in a variety of tissues [16], including

Table 7 Multivariate analysis of overall survival in prostate cancer patients

Variable	B	SE	Wald	*df*	*P*	Exp (B)
Tumor grade	1.433	0.701	5.253	1	0.035	3.476
Tumor Stage	1.575	0.527	15.217	1	0.002	4.612
IL18 -137	-1.673	1.132	4.076	1	0.073	0.180
IL18 -607	0.415	0.507	0.517	1	0.511	1.415

prostate cancer [17].

NANOG is known to control the differentiation of embryonic stem cells and plays a role in maintaining the self-repopulating ability [18]. A systematic study using animal models and *in vitro* cell systems demonstrated the key function of *NANOG* in human tumor development [19]. A recent study showed that the transforming growth factor β pathway is involved in regulating *NANOG* gene expression via binding to the *NANOG* proximal promoter [20]. Cultured human prostate cancer cells, prostate cancer xenografts, and primary prostate cancer cells express a functional variant of NANOG, *NANOG* mRNA, in cancer cells [19].

Cytokine IL-18 is known to play a critical role in the development and progression of tumors including prostate cancer. Our results revealed a strong association between increased expression of IL-18 and poor outcomes of patients with prostate cancer. Experimental studies demonstrated that IL-18 promote tumorigenesis, angiogenesis, and metastasis [21–22]. IL-18 is also known to induce multi-drug resistance to cancer cell lines [23]. Moreover, emerging evidence suggests that IL-18 has an important role in cancer stem cell phenotype and function. Additionally, IL-18 was found to enhance tumorigenicity in glioblastoma, which is consistent with the increased capacity of cancer stem cell self-renewal [24–25]. Consistent with these findings, our results showed that IL-18 might directly regulate the self-renewal capacity of cancer stem cells; however, the exact role of IL-8 in regulating CSC characteristics is not fully understood.

In the present study, we found no association between IL-18 and Nanog polymorphisms and a higher risk of prostate cancer. However, as in other studies [26–27], these polymorphisms were correlated with more advanced cancer stages. *IL-18* promoter polymorphisms have been associated with other cancers, including prostate and colorectal carcinomas [28–29], although the authors found no association between IL-18 and Nanog polymorphisms with cancer risk [30–31]. Our findings support the recent suggestion that the pleiotropic cytokine IL-18 has both anti-cancerous and pro-cancerous activities [32]. In fact, IL-18 activities are influenced by the tumor microenvironment. Thus, IL-18 can exert its antitumor activity by augmenting interferon-γ production, particularly in the presence of IL-12 [32]. However, recent data also suggested the pro-cancerous activity of this multifunctional cytokine under certain conditions depending on the tumor immune response at different tumor sites and probable genetic background [33]. In our patient group with prostate cancer, IL-18 and *NANOG* polymorphisms did not appear to be associated with prostate cancer susceptibility. This discrepancy may be attributable to the different genetic backgrounds and different environmental factors, such as the different carcinogens that initiate different cancers and different carcinogen exposures in the populations. Additionally, study design factors such as nonrandom sampling and the limited sample size should be considered. There also may have been selection bias in this hospital based, case-control study. Finally, we cannot exclude that the possibility that the association depends on a gene in linkage disequilibrium with the IL-18 gene or on the effect of IL-18 on another peptide. However, once the tumor appears, the highly productive IL-18 polymorphism promotes progression to a more advanced tumor grade, stage, etc. These results may be explained by the fact that IL-18 induces the production of angiogenic and growth factors [34–36].

We found that a genotype related to higher production of IL-18 is associated with a higher grade and stage of the tumor. IL-18 activates hypoxia-inducible growth factor [34] and vascular endothelial growth factor [35], and can activate angiogenesis in tumor nests [32]. Therefore, *IL-18* polymorphisms that increase its production would increase angiogenesis and provide adequate nutrients to transformed cells, promoting a more advanced stage. IL-18 is also correlated with disease progression. High-production polymorphisms in *IL-18* are associated with the dedifferentiation of tumor cells, leading to a more advanced tumor grade and stage grouping. Elevated IL-18 expression was found to correlate with the malignancy of skin cancers [37] and progression of breast cancer [26]. Therefore, IL-18 can directly promote proliferation by regulating proliferation stimulators. However, our findings revealed no association between prostate cancer risk and clinicopathological features. A recent study reported positive correlations between NANOG and oral cancer stem-like cells and high-grade oral squamous cell carcinoma [3]. Experimental evidence has also shown that knockdown of *NANOG* mRNA in cancer cells inhibits tumorigenesis and clonogenic growth of breast, colon, prostate, and gastrointestinal cancer cell lines [38–40]. Bioinformatic analysis showed that the rs1105786 polymorphism along with the polymorphisms in prostate cancer affected the splicing mechanism, suggesting a role for *NANOG* in tumorigenesis. No previous studies have evaluated the role of genetic variants in *NANOG* polymorphisms in prostate cancer. However, in this study, no significant association in *NANOG* rs1105786 polymorphism with tumor grade and stage was observed.

IL-18 was recently implicated in the migration of cancer and human melanoma cell lines through the generation of a region of interest and mitogen-activated protein kinase pathway [41]. Proinflammatory cytokines also induce adhesion receptors of endothelial cells for cancer cell attachment [42], which is necessary for blood-borne metastasis. Our results agree with these findings. The clinical importance of these parameters should be

investigated in patients with prostate cancer, particularly in those with bone metastasis. Additionally, larger sample sizes are needed. In the present study, polymorphisms related to IL-18 production were associated with the development of metastasis and lymph node involvement. However, polymorphisms related to Nanog were not associated with lymph node and metastasis involvement. Nevertheless, metastasis is a highly complex process that may involve numerous genes. This adds further complexity to the analysis of a specific polymorphism, as each gene is likely to contribute only moderately to the risk [43]. The association between overall survival and the IL-18-607 polymorphism was also analyzed. Because the median survival (50% mortality) was not achieved, we cannot confirm or rule out the statistical influence of this variable as a prognostic factor. Although polymorphisms related to IL-18 production were strongly correlated with more advanced stages of prostate cancer, explaining the tendency for an association with death risk (P = 0.076), Cox analyses revealed that *IL-18* and *Nanog* polymorphisms are not independent survival factors. *IL-18-137* and *Nanog* polymorphisms did not influence the risk of death in our patients. Thus, the influence of the IL-18-607 polymorphism may be more significant than that of IL-18-137, promoting higher-risk phenotypes, as also reported in nasopharyngeal carcinoma [32].

In conclusion, this study showed that *IL-18* promoter polymorphisms affect prostate cancer progression and prognosis. IL-18 is strongly correlated with higher tumor grade and stage, lymph node involvement, and distant metastasis. NANOG may be associated with the early stages of prostate cancer carcinogenesis. However, the molecular mechanism by which IL-18 and Nanog gene polymorphisms are associated with prostate cancer is unclear. Further studies are needed to determine whether IL-18 and Nanog polymorphisms are independent risk factors or indirect markers of different genetic and environmental factors. Additional studies may be useful for developing specific therapies against tumors based on the patient genotype.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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