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BRIEF REPORT

Association between genetic variant in hsa-miR-146a gene and prostate cancer progression: evidence from Serbian population

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Abstract
Purpose Two previous studies of association between rs2910164 in miR-146a gene and prostate cancer (PCa) risk have provided opposing results. Furthermore, no evidence of association of this SNP with standard prognostic parameters of PCa progression was obtained in mentioned studies. The main aim of this study was to evaluate the possible association between PCa onset and progression to a more aggressive form, since it has not been assessed in a population of European descent.
Methods In this study, 286 samples of peripheral blood were obtained from patients with PCa, while the control group comprised 199 volunteers derived from general population who gave samples of buccal swabs. For individuals diagnosed with PCa clinicopathological characteristics including serum prostate-specific antigen level at diagnosis, Gleason score (GS), and clinical stage were determined. Genotyping of rs2910164 was performed using Taqman® SNP Genotyping Assay. Analysis of SNP association was done using PLINK and SNPStats software.
Results rs2910164 showed no association with PCa risk. Nevertheless, heterozygous genotype was found to be associated with higher GS, as well as with the presence of distant metastases. rs2910164 was also shown to be associated with cancer aggressiveness ($p = 0.0067$; $OR_{GC} = 2.22$, 95 %CI 1.24–3.97; $OR_{CC} = 0.47$, 95 %CI 0.13–1.68).
Conclusions Our results show no evidence of association between rs2910164 and PCa risk in Serbian population. Conversely, this variant was found to be associated with PCa aggressiveness.

Keywords Association study · microRNA · miR-146a · Prostate cancer · Single-nucleotide polymorphism

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Introduction

Prostate cancer (PCa) is the most common cancer, and the third leading cause of cancer-related deaths among males in developed countries. Since incidence rates vary severely worldwide, PCa ranks sixth when considering cancer incidence and mortality rates among men in developing regions [1].

These alarming statistics have led to focusing research efforts on discovering molecular mechanisms underlying PCa onset and progression. Due to their diverse regulatory features, miRNA genes are emerging as potentially dysregulated and dysfunctional candidates in carcinogenesis in various tissues, including prostate [2–4]. Beside evaluating possible changes in miRNA and their precursors expression levels, with the intensification of association studies, several research groups focused on SNPs in miRNA genes and targets, mostly on those with potential functional implications. Two previous studies have provided inconsistent

results regarding association between SNP (rs2910164) in hsa-miR-146a gene and PCa risk [5, 6]. This variant is shown to affect biogenesis of mature miR-146a and is also located in the "seed" region of the passenger strand [5]. The study conducted by Xu et al. [5] in Chinese Han population has found that rs2910164 minor allele C confers reduced risk of PCa through affecting the amount of mature miR-146a. In contrast to these results, George et al. [6] have shown no evidence of association between this variant and the risk of PCa, nor with the several parameters of cancer progression in North Indian population.

Considering the observed differences, we tested the association between rs2910164 and PCa risk in Serbian population. Furthermore, we assessed the association of rs2910164 with standard prognostic parameters of PCa progression, as well as with the risk of cancer progression. To our knowledge, the present study is the first one assessing the association between variants in miR-146a and the risk of PCa conducted in a population of European descend.

Materials and methods

The study used peripheral blood samples obtained from 286 PCa patients treated in the period between 2009 and 2012 at Clinical Centers "Dr. Dragiša Mišović Dedinje" and "Zvezdara," Belgrade, Serbia. Research was conducted with the approval of ethics committees of these institutions. Written informed consents were obtained from participants before their inclusion in the study.

The control group comprised 199 healthy volunteers who gave samples of buccal swabs. The exclusion criteria for potential controls were the presence of any self-reported diseases and family history of PCa. Controls were recruited after passing standard annual physical examination. Mean ages for PCa patients and controls were 69.91 and 68.90 years, respectively. Diagnose of PCa was made using standard clinical procedure which included digital rectal examination, transrectal ultrasonography, abdominal and pelvic ultrasound, bone scintigraphy and radiography, serum prostate-specific antigen (PSA) level, and prostate biopsy. Serum PSA levels were determined by Hybritech method of monoclonal immunoassay. Clinical stage of cancer was determined according to TNM classification system. H&E-stained slides of paraffin-embedded prostate biopsy material were used to determine histological type of cancer and Gleason score (GS).

Patients with PCa were selected into groups based on the values of standard prognostic parameters—GS (GS <7 and GS ≥ 7) and clinical stage of localized tumor according to TNM classification system (T1/T2 and T3/T4). Two groups of patients were formed based on the presence of distant metastases. Based on the risk of cancer progression, two groups of patients were formed, according to Medeiros et al. [7]. The high-risk group included patients with GS ≥ 7, or advanced clinical stage (T3 and T4), or with bone metastases, while the low-risk group comprised patients with GS <7 and clinical stage T1 or T2.

Genomic DNA was isolated from peripheral blood and buccal swab samples using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturers' protocol. Genotypization of rs2910164 was performed using Taqman® SNP Genotyping Assay (Applied Biosystems, Foster City, California, USA). Statistical analysis of SNP association was done using PLINK [8] and SNPStats software [9]. Hardy–Weinberg equilibrium was assessed using exact test [10] implemented in PLINK software. Allelic and genotypic associations were evaluated by unconditional linear (for PSA in PCa patients) and logistic regression method. Genotype distributions among PCa patients with GS ≥ 7, tumor stage T3/T4, and metastatic PCa were compared to PCa patients with GS <7, tumor stage T1/T2, and without metastases, respectively. All the comparisons were done for three different genetic models: codominant, log-additive, and overdominant. The overdominant model was chosen based on results from other case–control studies involving the same genetic variant. Also, GC heterozygotes differ from both GG and CC homozygotes by producing three mature microRNAs: one from the leading strand and two from the passenger strand, each with its distinct set of target genes. Odds ratio (OR) was used as the effect size measure.

Results

The genotyping of SNP rs2910164 was successful for 199 control subjects and 286 PCa patients. Clinical and pathological characteristics of patients with PCa are presented in Table 1.

Allele and genotype frequencies of rs2910164 in PCa patients, as well as in the control group, are summarized in Table 2. Genotype distributions were consistent with Hardy–Weinberg equilibrium among controls (results not shown). The comparison of genotype frequencies in PCa patients and controls yielded no evidence of association between rs2910164 and the risk of PCa (Table 2).

The analysis of association between rs2910164 and the serum PSA level at diagnosis, as well as between this variant and the stage of localized tumor, did not show statistical significance ($p = 0.68$ and $p = 0.22$, for codominant model, respectively) (results not shown). Conversely, when comparing genotype distributions in subgroups of PCa patients with GS ≥ 7 and GS <7, statistically significant difference was found for both codominant and overdominant genetic

Table 1 Classification of patients with prostate cancer based on the values of standard prognostic parameters of disease progression, presence of metastases, and the risk of cancer progression

Characteristics	Prostate cancer patient's *n* (%)
PSA at diagnosis	
<10 ng/ml	88 (30.99)
10–20 ng/ml	75 (26.41)
>20 ng/ml	121 (42.60)
Unknown	2
Gleason score	
4	7 (2.55)
5	15 (5.47)
6	138 (50.36)
7	73 (26.64)
8	25 (9.12)
9	13 (4.74)
10	3 (1.09)
Unknown	12
Clinical stage	
T1	44 (18.11)
T2	122 (50.20)
T3/T4	77 (31.69)
Unknown	43
Metastases	
Distant (M+)	45 (16.36)
Regional (N+) or not detected	230 (83.64)
Unknown	30
Aggressiveness	
Low	103 (39.16)
High	160 (60.84)
Unknown	23

PSA prostate-specific antigen

Table 2 Association of rs2910164 with prostate cancer risk

Genetic model	No of PCa patients (%)	No of controls (%)	PCa versus controls	
			OR (95 % CI)[a]	*p* value[a]
Codominant				
GG	184 (64.3)	129 (64.8)	1.00	0.89
GC	90 (31.5)	63 (31.7)	1.01 (0.68–1.49)	
CC	12 (4.2)	7 (3.5)	1.27 (0.48–3.33)	
Overdominant				
GG + CC	196 (68.5)	136 (68.3)	1.00	0.98
GC	90 (31.5)	63 (31.7)	0.99 (0.67–1.47)	
Log-additive				
–	–	–	1.05 (0.76–1.45)	0.76

PCa prostate cancer, *OR* odds ratio

[a] Adjusted for age

models ($p = 0.0096$ and $p = 0.045$, respectively) (Table 3). Under codominant model, heterozygotes were found to be associated with higher GSs, while CC homozygotes have shown the opposite effect ($OR_{GC} = 1.57$, 95 %CI 0.93–2.66; $OR_{CC} = 0.15$; 95 %CI 0.02–1.17). Men with GC genotype were found to have an increased risk of higher GS when compared to men with homozygous genotype (OR = 1.70, 95 %CI 1.01–2.87).

When PCa patients without detected metastatic disease were compared with patients with distant metastases marginal significance was obtained for association between rs2910164 and progression of localized to advanced metastatic PCa under assumed overdominant genetic model ($p = 0.05$; OR = 1.95, 95 %CI 1.01–3.79) (Table 3).

Carriers of GC genotype were found to have a 2.22-fold increased risk of developing more aggressive PCa, when compared to men with GG genotype, while for CC genotype opposite direction of association was shown ($p = 0.0067$; $OR_{GC} = 2.22$, 95 %CI 1.24–3.97; $OR_{CC} = 0.47$, 95 %CI 0.13–1.68, codominant model) (Table 3). Furthermore, when assuming overdominant genetic model, heterozygous carriers of rs2910164 allele C were shown to have 2.32-fold increased risk of aggressive PCa, compared to carriers of both homozygotes ($p = 0.0033$; 95 %CI 1.30–4.13).

Discussion

A SNP located in has-miR-146a has been analyzed for association with various cancers, including PCa. It was shown that this SNP influences the biogenesis of mature miRNA and resides within the sequence encoding mature passenger strand of miR-146a (miR-146a*), specifically its "seed" region [5]. Therefore, its potential influence on the neoplastic transformation process could be attributed to affecting biosynthesis of mature miR-146a, which was found to be upregulated in multiple cancers, and/or to the change of passenger strand target specificity, assuming that this mature form of miR-146a is functional [5, 11]. Previous studies have yielded evidence of association between rs2910164 and the risk of papillary thyroid, breast, ovarian, gastric, and several other types of cancer [11–16]. As for PCa, studies conducted in Han Chinese and North Indian populations have provided inconclusive evidence of association between the risk of developing this malignancy and rs2910164 [5, 6]. Xu et al. [5] have shown that carriers of C allele have the reduced risk of PCa, with the GC and CC genotype ORs for developing PCa of 0.71 and 0.50, respectively, compared with GG homozygotes. In contrast with these results, George et al. [6] have found no evidence of association between rs2910164 and the risk of PCa, but in their study genotype distributions deviated significantly from HWE. We intended to elucidate potential association

Table 3 Association of rs2910164 with Gleason score, presence of metastases, and tumor stage

Genetic model	Gleason score (GS ≥7 vs GS <7)			
	GS <7 (%)	GS ≥7 (%)	OR (95 % CI)[a]	*p* value[a]
Codominant				
GG	107 (66.9)	69 (61.1)	1	**0.0096***
GC	42 (26.2)	43 (38)	1.57 (0.93–2.66)	
CC	11 (6.9)	1 (0.9)	0.15 (0.02–1.17)	
Overdominant				
GG + CC	118 (73.8)	70 (62)	1	**0.045**
GC	42 (26.2)	43 (38)	**1.70 (1.01–2.87)**	
Log-additive				
–	–	–	1.00 (0.66–1.53)	0.99
Genetic model	Presence of distant metastases			
	Present (%)	Absent (%)	OR (95 % CI)[a]	*p* value[a]
Codominant				
GG	157 (68.3)	24 (53.3)	1	0.14
GC	64 (27.8)	19 (42.2)	**1.99 (1.02–3.89)**	
CC	9 (3.9)	2 (4.4)	1.31 (0.26–6.54)	
Overdominant				
GG + CC	166 (72.2)	26 (57.8)	1	**0.05**
GC	64 (27.8)	19 (42.2)	**1.95 (1.01–3.79)**	
Log-additive				
–			1.55 (0.91–2.63)	0.11
Genetic model	High versus low aggressiveness[b]			
	Low (%)	High (%)	OR (95 % CI)[a]	*p* value[a]
Codominant				
GG	75 (72.8)	96 (60)	1	**0.0067***
GC	21 (20.4)	60 (37.5)	**2.22 (1.24–3.97)**	
CC	7 (6.8)	4 (2.5)	0.47 (0.13–1.68)	
Overdominant				
GG + CC	82 (79.6)	100 (62.5)	1	**0.0033**
GC	21 (20.4)	60 (37.5)	**2.32 (1.30–4.13)**	
Log-additive				
–			1.32 (0.85–2.07)	0.22

OR odds ratio, *GS* Gleason score

[a] Adjusted for age

[b] The high-risk group included patients with GS ≥7, or clinical stage T3 or T4, or with bone metastases, while the low-risk group comprised patients with GS <7 and clinical stage T1 or T2

* Statistically significant results are shown in bold

of this variant and PCa risk in Serbian population, which is the first population of European descent in which the mentioned association was tested and found no evidence to support this assumption.

Our results obtained in Serbian population significantly differ from previously reported from Chinese Han [5] and North Indian population [6], since we have found evidence for association of this SNP with GS, while for association with the presence of distant metastases marginal statistical significance was reached. In contrast to results obtained for CC genotype, rs2910164 GC heterozygote was found to confer increased risk of developing tumor with GS ≥7 among PCa patients. For several comparisons, association under overdominant model was found to be significant, which possibly reflects the combined effect of heterozygosity on miRNA biogenesis and target selection, since heterozygous genotype is the only one potentially producing three different mature miRNAs with different target specificity. The importance of rs2910164 heterozygotes for cancerogenesis was previously proposed by Jazdzewski et al. [11, 17]. Furthermore, association between rs2910164 and PCa aggressiveness was found to be highly statistically significant. Therefore, data obtained in the present study suggest possible involvement of rs2910164 and miR-146a in PCa progression. Although this study was limited by the lack of data on miR-146a expression in normal and malignant prostate tissue, the above-mentioned assumptions are substantiated by the previous expression analyses

that demonstrated the loss of miR-146a in hormone-refractory PCa and the effect of expression of this miRNA on tumorigenicity and angiogenesis [18, 19].

The main limitation of this study is a relatively small number of participants. Therefore, in order to make further conclusions about the association between rs2910164 and the risk of PCa, as well as about possible involvement of miR-146a and molecular pathogenesis of PCa, study population needs to be enlarged. Discordance in results of previous and the present study can be explained by differences in ethnical backgrounds that are clearly reflected in significant departure in allele frequencies. Further analysis involving a larger sample and in other populations should be performed in order to confirm the association of rs2910164 with the risk of PCa progression to a more aggressive form.

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Conflict of interest The authors declare that they have no conflict of interest.

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