Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2024.71508

Turk J Obstet Gynecol 2024;21:57-63

Investigation of *PD-1* gene variants in patients with endometrial cancer: A case-control study

Endometriyum kanserli hastalarda PD-1 gen varyantlarının araştırılması: Olgu kontrol çalışması

Mohammad Javad Fattahi[1], Mozhdeh Momtahan[2], Maryam Poostkar[1], Zahra Shiravani[2,3], Nasrollah Erfani[1], Mohammad Reza Haghshenas[1], Masoumeh Hashemi[2], Abbas Ghaderi[1], Ali Kashkooe[1]

[1]*Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[2]*Gynecology Oncology Division, Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[3]*Maternal-fetal Medicine Research Center, Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

Abstract

Objective: To assess the possible association of two single-nucleotide polymorphisms (SNPs), PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T), with endometrial cancer (EC) susceptibility. In addition, the correlations between these SNPs and available clinicopathologic characteristics of patients with EC were investigated.

Materials and Methods: In this case-control study, 147 women with pathologically confirmed EC and 258 age- and ethnically matched healthy women were enrolled between June 2019 and May 2022. Genomic DNA was extracted, and genotyping of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) SNPs was performed. Haplotype analysis was also performed. Pearson's chi-square test with Yates correction was used to evaluate differences in allele and genotype distributions. The 95% confidence interval and odds ratio were determined using an unconditional logistic regression model.

Results: There were no remarkable differences in the allele and genotype distributions of PD-1.3 (rs11568821) and PD-1.5 (rs2227981) between healthy controls and EC patients. However, there was a remarkable difference in the AC haplotype between the control and EC groups. No association was found between the investigated SNPs and the clinicopathologic features of EC.

Conclusion: Our results indicated that the aforementioned SNPs were not related to the risk of EC in the southern Iranian population.

Keywords: Endometrial cancer, programed cell death-1, polymorphism, single-nucleotide polymorphisms

Öz

Amaç: Tek nükleotid polimorfizmlerinden (SNP) PD-1,3 (+7146G/A-rs11568821) ve PD-1,5 (+7785C/T-rs2227981 ile endometriyal kanser (EK) duyarlılığı arasındaki olası ilişkiyi değerlendirmektir. Ayrıca bu SNP'ler ile EK'li hastaların mevcut klinikopatolojik özellikleri arasındaki korelasyonlar araştırıldı.

Gereç ve Yöntemler: Bu olgu-kontrol çalışmasına Haziran 2019 ile Mayıs 2022 arasında patolojik olarak doğrulanmış EK'li 147 kadın ve yaş ve etnik açıdan uyumlu 258 sağlıklı kadın dahil edildi. Genomik DNA çıkarıldı ve PD-1,3 (rs11568821) ve PD-1,5 (rs2227981) SNP'lerinin genotiplemesi yapıldı. Haplotip analizi de yapıldı. Alel ve genotip dağılımlarındaki farklılıklar, Yates düzeltmeli Pearson ki-kare testi kullanılarak değerlendirildi. %95 güven aralığını ve olasılık oranını hesaplamak için koşulsuz bir lojistik regresyon modeli kullanıldı.

Bulgular: Tek nükleotid polimorfizmlerinden PD-1,3 (rs11568821) ve PD-1,5 (rs2227981) alel ve genotip dağılımları açısından EK'li hastalar ve sağlıklı kontroller arasında dikkate değer bir fark yoktu. Ancak EK'li hastalar ile kontrol grubu arasında AC haplotipinde dikkate değer bir fark vardı. Araştırılan SNP'ler ile EK'nin klinikopatolojik özellikleri arasında da bir ilişki bulunamadı.

Sonuç: Sonuçlarımız yukarıda bahsedilen SNP'lerin İran toplumunda EK riski ile ilişkili olmadığını gösterdi.

Anahtar Kelimeler: Endometriyum kanseri, programlanmış hücre ölümü-1, polimorfizm, tek nükleotid polimorfizmleri

PRECIS: Our results indicated that *PD-1* gene variants (PD-1.3 and PD-1.5) were not associated with the risk of endometrial cancer in the Iranian population.

Address for Correspondence/Yazışma Adresi: Mohammad Javad Fattahi PhD,
Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Phone: +98(0)71 32303687 **E-mail:** mfattahi@sums.ac.ir **ORCID ID:** orcid.org/0000-0002-5007-242X

Received/Geliş Tarihi: 26.03.2024 **Accepted/Kabul Tarihi:** 28.04.2024

Introduction

Endometrial cancer (EC), which originates from the epithelium of the uterus, is the fourth most frequent malignancy in women worldwide and the most common cancer of the female reproductive system[1]. During the last two decades, mortality rates and the incidence of EC have increased. Thus, EC is a major concern for women's health, especially in developed countries. About 65,000 new cases of EC and 12,000 deaths are expected in the United States in 2022[2]. Although EC mainly affects postmenopausal women 60-70 years of age, approximately 5-10% of cases experience it under 40 years of age[3]. EC is divided into several molecular subtypes that show distinct clinical and pathological behavior[4]. Genetic alterations and dysregulated immune responses determine the risk level and prognosis of EC patients[5]. Despite current improvements in therapeutic protocols for other gynecologic malignancies, few improvements are available for the management of advanced-stage EC. Therefore, a deep understanding of the molecular changes associated with EC is needed to identify new biomarkers for the early diagnosis of EC and to identify new targets for prevention and more effective therapeutic approaches[5].

Programed cell death-1 (PD-1, CD279), a type I transmembrane glycoprotein, is one of the most important immune checkpoints belonging to the CTLA-4/CD28 subfamily of the immunoglobulin (Ig) superfamily. It is encoded by the *PDCD1* gene, which is located on chromosome 2q37.3[6] and is a co-inhibitory receptor that downregulates the activation of T-cells and leads to the maintenance of peripheral tolerance. It is expressed on activated immune cells, including B cells, $CD8^+$ and $CD4^+$ T-cells, Natural killer T-cells, regulatory T-cells (Treg), monocytes, and some DC subsets. PD-1 is also a marker of exhausted T lymphocytes[6,7]. PD-1 ligands (PD-L1/2) are expressed on a broad range of human hematopoietic and non-hematopoietic cells, as well as tumor cells. When PD-1 binds to its ligands, it induces inhibitory signals that suppress cytokine production and T-cell proliferation and attenuate tumor immunity[6]. Recent studies have revealed that antibodies that block immune checkpoints, such as anti-PD-1/PD-L1, are one of the most promising immunotherapy approaches for the treatment of some refractory tumors[8]. Despite the clinical success of immune checkpoint inhibitor therapies, some patients with EC do not respond well to these treatments; therefore, markers predicting the efficacy of anti-PD-1/PD-L1 immunotherapy may aid in patient selection and decision making by differentiating responders from non-responders[9].

One of the most frequent sources of genetic diversity in the human genome is single-nucleotide polymorphisms (SNPs). Based on where SNPs are located, within gene sequences or in regulatory regions near a gene, they might have different outcomes at the phenotypic level[10]. They can also be considered as molecular markers in association studies related to complicated human diseases such as autoimmune diseases and cancer. Genetic polymorphisms may affect *PDCD1* and *PD-L1* gene expression[7,11]. Previous studies have found that some PD-1 functional SNPs are related to different types of cancer, including brain tumors, thyroid cancer, colon cancer, and gastric cancer[12-15]. However, there are also some conflicting results[16,17].

Therefore, in this study, we evaluated the possible association of two known SNPs in the *PDCD1* gene, PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T), with EC susceptibility in a southern Iranian population. In addition, correlations between these SNPs and existing clinicopathologic features of the patients were evaluated.

Materials and Methods

Study Population

In this case-control study, we selected 147 women with pathologically confirmed EC as a case group who were enrolled at Shahid Faghihi Hospital affiliated with Shiraz University of Medical Sciences (Shiraz, Iran) between June 2019 and May 2022. All patients with EC were staged using the International Federation of Gynecology and Obstetrics (FIGO) staging criteria. The control group included 258 age, ethnically matched healthy women who were selected from blood donors referred to the Fars Blood Transfusion Organization (Shiraz, Iran) without any history or evidence of clinical problems, especially gynecological disorders, autoimmune diseases, and cancer, and without any history of medication as inclusion criteria. The Shiraz University of Medical Sciences Research Ethics Committee approved this study (approval number: IR.SUMS.REC.1398.1160, date: 28.12.2019).

DNA Extraction and Molecular Analysis

After written informed consent, 4 mL of peripheral blood was obtained from healthy women and EC patients in tubes containing EDTA. QIAamp DNA Mini Kit (Qiagene, Germany) was used to extract genomic DNA. Genotyping of PD-1.3 (+7146G/A-rs11568821) and PD-1.5 (+7785C/T-rs2227981) SNPs was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using specific primers and *Pst1* (Fermentas, Lithuania) and *PvuII* (Fermentas, Lithuania) restriction enzymes, respectively (Table 1). The digested products were separated by electrophoresis on an agarose gel (3%) stained with a safe stain for visualization under UV light.

Statistical Analysis

Haplotype analysis and deviation from Hardy-Weinberg equilibrium were assessed using the Arlequin software package algorithms. The SPSS software package (version 20, Chicago, IL, USA) was used to analyze the data. Differences in allele and genotype frequencies were calculated using Pearson's chi-square test with Yates correction. An unconditional logistic regression model was used to calculate the 95% confidence interval (CI) and odds ratio (OR). $P<0.05$ was statistically significant.

Results

Study Population

The demographic and clinicopathological data of the 147 patients with EC and 258 healthy controls are presented in Table 2. There were no remarkable differences between the mean age (p=0.26), age at menarche (p=0.07), age at menopause (p=0.38), and body mass index (p=0.09) of healthy controls and EC patients. The tumor type in 104 (70.4%) out of 147 EC patients was endometrioid adenocarcinoma, 124 (84.3%) of the EC patients were in FIGO stage I, and 82 (55.7%) EC patients were diagnosed with grade I carcinoma. The prevalence of diabetes and hypertension in patients with EC was 20 (13.6%) and 34 (23.1%), respectively.

PDCD1 Gene Variants and the Risk of EC

In this study, genotype distribution at positions PD-1.5 (rs2227981) and PD-1.3 (rs11568821) in both controls and EC patients was in Hardy-Weinberg equilibrium. As presented in Table 3, the frequencies of PD-1.3 (rs11568821) genotypes were 112 (76.2%) for GG, 33 (22.5%) for GA, and 2 (1.3%) for AA out of 147 patients, and in controls, there were 204 (79.1%) GG, 50 (19.4%) GA, and 4 (1.5%) AA out of 258 participants. Our results showed no remarkable differences in the frequencies of PD-1.3 alleles and genotypes between healthy controls and patients, and PD-1.3 (rs11568821) did not change the overall risk of EC overall (Table 3).

The frequencies of PD-1.5 (rs2227981) genotypes were 65 (44.2%) for CC, 61 (41.6%) for CT, and 21 (14.3%) for TT. In controls, the frequencies were 109 (42.2%) for CC, 107 (41.5%) for CT, and 42 (16.3%) for TT, with no remarkable differences between the two groups (Table 3). Statistical analysis also showed no remarkable differences in the allele frequency of PD-1.5 (rs2227981) between cases and healthy controls (Table 3).

Table 1. Primer sequences and PCR-RFLP conditions for amplification of *PDCD1* gene

Locus	Primer sequence	Annealing temperature	RE	Length of digested fragments
PD-1.3 (+7146G/A- rs11568821)	F: 5′-CCAGGCAGCAACCTCAATC-3′ R: 5′-GGTGTCCCCAGATCACACAG-3	58 °C	Pst1	G: 381 bp A:277 bp, 104 bp
PD-1.5 (+7785C/T-rs2227981)	F: 5′-GACGGAGTATGCCACCATTGTC-3′ R: 5′-AAATGCGCTGACCCGGGCTCAT- 3′	58 °C	PvuII	C: 196 bp T: 125 bp, 71 bp

RE: Restriction enzyme, RFLP: Restriction fragment length polymorphism, PCR: Polymerase chain reaction

Table 2. Demographic and clinicopathologic information of the study population

Variables		EC patients (n=147)	Healthy controls (n=258)	p-value
Age, mean ± SD		57.42±10.86	55.43±8.36	0.26
Age at menopause, mean ± SD		50.92±4.59	51.51±2.39	0.38
Age at menarche, mean ± SD		12.16±1.33	12.59±1.61	0.07
BMI, kg/m^2		30.25±5.79	28.8±3.67	0.09
Diabetes, n (%)		20 (13.6%)	-	-
Hypertension, n (%)		34 (23.1%)	-	-
Histological grade	I	82 (55.7%)	-	
	II	35 (23.8%)	-	
	III	30 (20.4%)	-	
FIGO stage	I	124 (84.3%)	-	
	II	9 (6.1%)	-	
	III	14 (9.5%)	-	
	IV	-	-	
Lymph node involvement	Yes No	22 (14.9%) 125 (85.0%)	- -	
Tumor size	≥3 cm ≤3 cm	60 (40.8%) 87 (59.2%)	- -	

EC: Endometrial cancer, FIGO: International Federation of Gynecology and Obstetrics, SD: Standard deviation, BMI: Body mass index

The allele and genotype frequencies of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) were analyzed according to the clinicopathological features of patients with EC. The results showed that neither of the two SNPs was associated with any of the clinicopathological features of the disease, including lymph node (LN) involvement status, stage, histological grade, and tumor size.

PD-1 Haplotype Distributions in Controls and EC Patients

GC, GT, AC, and AT haplotypes were obtained from PD-1 SNPs using algorithms from the Arlequin software package. The GC haplotype was the most common haplotype in both EC patients (58.50%) and healthy controls (55.81%). Statistical analysis revealed that AT, GC, and GT haplotype distributions were not associated with EC (Table 4). At the same time, it was found that the AC haplotype frequency was remarkably different in EC patients compared with controls (Table 4). This haplotype was found to play a protective role in the development of EC (OR=0.57, 95% CI=0.33-0.96, p=0.04) (Table 4).

Discussion

In this study, we did not detect remarkable differences in the allele and genotype distributions of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) between the control group and EC patients. However, there was a remarkable difference in the AC haplotype between healthy controls and EC patients. The results also showed no association between the evaluated SNPs and the clinicopathological features of EC.

Several studies regarding the association of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) with the risk and/or progression of various types of cancer have yielded inconsistent results. Haghshenas et al.[17] could not find an association between PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) and the risk of breast cancer in the Iranian population. Their results also showed no correlation between the evaluated genotypes and clinicopathologic features of breast cancer. Furthermore, another study by Piredelkhosh et al.[16] found that the SNPs mentioned above had no remarkable association with non-small-cell lung cancer (NSCLC) susceptibility. Li et al.[18] also demonstrated no remarkable association between PD-1.5 (+7785C/T) and the risk of ovarian cancer. In contrast, several recent studies have revealed an association between *PDCD1* gene variants, both in terms of genotypic and allelic frequencies, and different types of cancer[19].

The human genome contains nearly 10 million SNPs. Some SNPs play a role in susceptibility to environmental factors,

Table 3. Genotype and allele frequencies of PD-1 SNPs in EC patients and healthy controls

SNPs	Genotype/allele	Healthy controls n (%)	Patients n (%)	p-value	OR	95% CI
PD1.3	GG	204 (79.1%)	112 (76.2%)	1.0	1.0	Reference
	GA	50 (19.4)	33 (22.5%)	0.41	0.83	0.53-1.29
	AA	4 (1.5%)	2 (1.3%)	0.90	1.04	0.48-2.29
	G	458 (88.75%)	257 (87.4%)	1.0	1.0	Reference
	A	58 (11.25%)	37 (12.6%)	0.56	0.93	0.75-1.16
PD1.5	CC	109 (42.2%)	65 (44.2%)	1.0	1.0	Reference
	CT	107 (41.5%)	61 (41.6%)	0.82	1.05	0.70-1.55
	TT	42 (16.3%)	21 (14.3%)	0.53	1.09	0.82-1.44
	C	325 (63.0%)	191 (65.0%)	1.0	1.0	Reference
	T	191 (37.0%)	103 (35.0%)	0.57	1.04	0.89-1.21

OR: Odds ratio, CI: Confidence interval, SNPs: Single nucleotide polymorphisms, EC: Endometrial cancer

Table 4. PD-1 haplotype distributions in EC patients and healthy controls

Haplotypes		Patients n (%)	Healthy controls n (%)	p-value	OR	95% CI
PD1.3	PD1.5					
G	C	86 (58.50%)	144 (55.81%)	0.45	1.03	0.80-1.31
G	T	49 (33.45%)	85 (32.94%)	0.27	1.13	0.85-1.47
A	C	11 (7.50%)	26 (10.07%)	0.04	0.57	0.33-0.96
A	T	1 (0.69%)	3 (1.16%)	0.80	0.76	0.24-2.56

OR: Odds ratio, CI: Confidence interval, EC: Endometrial cancer, *p<0.05

including toxins. Others increase the risk of developing certain diseases, affect a patient's response to certain medications, and are associated with some complex diseases such as cancer[7]. Recent genome-wide association studies have implicated that SNPs in genes that encode immunoregulatory molecules are involved in the inability of immune responses to control tumor growth and thus contribute to the risk of developing various types of tumors. They contribute to the molecular pathogenesis of complex diseases through various functional mechanisms[20]. EC is the most prevalent gynecological cancer in developed countries. SNPs within different genes are involved in endometrial carcinogenesis. In a review study by Bafligi et al.[21], SNPs in KLF, SOX4, HNF1B, CYP19A1, EIF2AK, and MYC were found to be closely associated with EC. In the current study, the association between two SNPs in the *PDCD1* gene (PD-1.5 and PD-1.3) and EC was evaluated.

rs11568821 (PD-1.3 G/A) is localized within intron 4 of the *PDCD1* gene[12]. The rs11568821 (PD-1.3 G/A) polymorphism can alter the expression of the *PDCD1* gene through a substitution of A for G, which may result in a loss of PD-1 inhibitory functions in individuals carrying the PD-1.3 A allele[20]. Parakh et al.[22] reported that melanoma patients with the GG genotype of PD-1.3 had more complete responses than those with the AG genotype, and the G allele remarkably correlated with a longer median progression-free survival than the A allele. However, our results did not show any remarkable differences in the frequency of PD-1.3 alleles and genotypes between the control group and EC patients. In other words, the PD-1.3 polymorphism did not modify the overall risk of EC. In line with our results, a recent study also reported no remarkable association between PD-1.3 and NSCLC[16]. Another study demonstrated a trend toward an association of PD-1.3 genotypes with skin basal cell carcinoma, although this association was not remarkably significant[23]. Furthermore, no significant association was found between hepatocellular carcinoma and breast cancer in a Turkish population[24] and an Iranian population[17], respectively. However, the PD-1.3 polymorphism was correlated with colorectal cancer in the Iranian population[25].

Another *PDCD1* gene polymorphism investigated in this study was rs2227981 (PD-1.5 C/T). It is located in exon 5 of PDCD1 and is a synonymous SNP. Because of the linkage disequilibrium between the rs2227981 (PD-1.5 C/T) polymorphism and other *PDCD1* gene polymorphisms, PD-1.5 may affect *PDCD1* expression at the mRNA and protein levels[7]. The possible association between the rs2227981 (PD-1.5 C/T) polymorphism and the risk of developing cancer was evaluated in three meta-analyses. The results showed that the T allele of the rs2227981 (PD-1.5 C/T) polymorphism remarkably reduced susceptibility to cancer[26-28]. The results from the Chinese Han population also suggested that PD-1.5 was potentially related to NSCLC susceptibility[29], and the results from the Iranian population demonstrated its association with gastric cancer risk. However, our findings revealed no remarkable differences between controls and EC patients regarding the allele and genotype distribution of PD-1.5. Consistent with our study, Ma et al.[30] and Fathi et al.[20] failed to show a PD-1.5 association with the risk of NSCLC and head and neck squamous cell carcinoma (HNSCC), respectively, and Li et al. could not show a PD-1.5 association with ovarian cancer in the Chinese population[18]. The inconsistency in results may be due to differences in the molecular pathology of the diseases studied and/or differences in minor allele frequency (MAF) in different populations.

Aside from the above findings, our investigation showed that none of the two SNPs (PD-1.3 and PD-1.5) correlated with any of the clinicopathological features of EC patients, including LN involvement status, tumor size, stage, and histological grade. In line with our results, another study showed no association between the investigated SNPs and tumor size, tumor grade, tumor stage, LN involvement, or other clinicopathologic characteristics of breast cancer[17]. Moreover, Li et al.[18] found that *PDCD1* gene polymorphisms may be associated with the development of epithelial ovarian cancer but not with its clinical outcome in these patients. In addition, although our statistical analysis revealed that the AT, GC, and GT haplotype distributions were not associated with EC, the AC haplotype frequency was remarkably different in patients with EC compared with controls. This haplotype was found to play a protective role in the development of EC. Another study by Fathi et al.[20] suggested that although *PDCD1* gene polymorphisms at positions PD-1.5 and PD-1.3 did not correlate with HNSCC susceptibility, haplotype combinations resulting from these polymorphisms may confer susceptibility. In contrast, in a previous study evaluating four haplotypes derived from PD-1.5 and PD-1.3 polymorphisms in an Iranian population, no differences in haplotype distributions were observed between breast cancer patients and the control group[17].

Study Limitations

There are several limitations to our study that need to be considered when interpreting the results. First, only two functional SNPs in the *PDCD1* gene were selected to evaluate their associations with EC susceptibility, which may not reflect the effect of all genetic variants in *PDCD1*. Second, the sample size was relatively small. To elucidate the exact role of *PDCD1* gene polymorphisms in the pathogenesis of EC, it is necessary to study the full range of PD-1 genetic variants, perform a complete haplotype analysis in a larger sample size and in different ethnic groups, and perform a functional study of the haplotypes that emerge.

Conclusion

This study offered insight into the roles of PD-1.5 and PD-1.3 polymorphisms in the etiology of EC and their association with the clinicopathological features of patients with EC. The

results showed that these SNPs are not related to the risk of EC in the southern Iranian population. Current studies have shown that managing EC can be challenging; therefore, a profound knowledge of genetic variation and the mechanisms of its pathogenesis will lead to the achievement of therapeutic and diagnostic precision in this complicated cancer, which continues to increase in incidence and mortality.

Ethics

Ethics Committee Approval: The Shiraz University of Medical Sciences Research Ethics Committee approved this study (approval number: IR. SUMS. REC.1398. 1160, date: 28.12.2019).

Informed Consent: All participants provided informed consent before entering the study.

Authorship Contributions

Surgical and Medical Practices: M.M., Z.S., M.R.H., Concept: M.J.F., N.E., A.G., Design: M.J.F., M.M., N.E., A.G., Data Collection or Processing: Z.S., M.R.H., M.H., A.K., Analysis or Interpretation: M.J.F., M.P., N.E., M.R.H., M.H., A.K., Literature Search: M.J.F., M.P., M.H., A.K., Writing: M.J.F., M.M., M.P., Z.S., M.R.H., A.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7-34.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022;72:7-33.
3. Lai C-H, Wang C-J, Chao A. The clinical management of endometrial cancer in young women. Curr Obstet Gynecol Rep 2013;2:26-31.
4. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. Lancet 2016;387:1094-108.
5. Liu Y. Immune response characterization of endometrial cancer. Oncotarget 2019;10:982-92.
6. Salmaninejad A, Valilou SF, Shabgah AG, Aslani S, Alimardani M, Pasdar A, et al. PD-1/PD-L1 pathway: Basic biology and role in cancer immunotherapy. J Cell Physiol 2019;234:16824-37.
7. Salmaninejad A, Khoramshahi V, Azani A, Soltaninejad E, Aslani S, Zamani MR, et al. PD-1 and cancer: molecular mechanisms and polymorphisms. Immunogenetics 2018;70:73-86.
8. Jindal V, Gupta S. Expected paradigm shift in brain metastases therapy-Immune checkpoint inhibitors. Mol Neurobiol 2018;55:7072-8.
9. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med 2018;50:1-11.
10. Fareed M, Afzal M. Single nucleotide polymorphism in genome-wide association ofhuman population: a tool for broad spectrum service. Egypt J Med Hum Genet 2013;14:123-34.
11. Yarchoan M, Albacker LA, Hopkins AC, Montesion M, Murugesan K, Vithayathil TT, et al. PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight 2019;4:e126908.
12. Namavar Jahromi F, Samadi M, Mojtahedi Z, Haghshenas MR, Taghipour M, Erfani N. Association of PD-1.5 C/T, but Not PD-1.3 G/A, with Malignant and Benign Brain Tumors in Iranian Patients. Immunol Invest 2017;46:469-80.
13. Haghshenas MR, Dabbaghmanesh MH, Miri A, Ghaderi A, Erfani N. Association of PDCD1 gene markers with susceptibility to thyroid cancer. J Endocrinol Invest 2017;40:481-6.
14. Mojtahedi Z, Mohmedi M, Rahimifar S, Erfani N, Hosseini SV, Ghaderi A. Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with colon cancer. Gene 2012;508:229-32.
15. Savabkar S, Azimzadeh P, Chaleshi V, Nazemalhosseini Mojarad E, Aghdaei HA. Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with gastric cancer. Gastroenterol Hepatol Bed Bench 2013;6:178-82.
16. Piredelkhosh Z, Kazemi T, Haghshenas MR, Ghayumi MA, Erfani N. Investigation of Programmed Cell Death-1 (PD-1) Gene Variations at Positions PD1.3 and PD1.5 in Iranian Patients with Non-small Cell Lung Cancer. Middle East J Cancer 2018;9:13-7.
17. Haghshenas MR, Naeimi S, Talei A, Ghaderi A, Erfani N. Program death 1 (PD1) haplotyping in patients with breast carcinoma. Mol Biol Rep 2011;38:4205-10.
18. Li Y, Zhang H-l, Kang S, Zhou RM, Wang N. The effect of polymorphisms in PD-1 gene on the risk of epithelial ovarian cancer and patients' outcomes. Gynecol Oncol 2017;144:140-5.
19. Wen Y, Liu J, Su Y, Chen X, Hou Y, Liao L, et al. Forensic biogeographical ancestry inference: recent insights and current trends. Genes Genomics 2023;45:1229-38.
20. Fathi F, Faghih Z, Khademi B, Kayedi T, Erfani N, Gahderi A. PD-1 haplotype combinations and susceptibility of patients to squamous cell carcinomas of head and neck. Immunol Invest 2019;48:1-10.
21. Bafligil C, Thompson DJ, Lophatananon A, Smith MJ, Ryan NA, Naqvi A, et al. Association between genetic polymorphisms and endometrial cancer risk: a systematic review. J Med Genet 2020;57:591-600.
22. Parakh S, Musafer A, Paessler S, Witkowski T, Suen CSNLW, Tutuka CSA, et al. PDCD1 polymorphisms may predict response to anti-PD-1 blockade in patients with metastatic melanoma. Front Immunol 2021;12:672521.
23. Fathi F, Ebrahimi M, Eslami A, Hafezi H, Eskandari N, Motedayyen H. Association of programmed death-1 gene polymorphisms with the risk of basal cell carcinoma. Int J Immunogenet 2019;46:444-50.
24. Bayram S, Akkız H, Ülger Y, Aynur Bekar A, Akgöllü E, Yıldırım S. Lack of an association of programmed cell death-1 PD1. 3 polymorphism with risk of hepatocellular carcinoma susceptibility in Turkish population: a case–control study. Gene 2012; 511:308-13.
25. Yousefi AR, Karimi MH, Shamsdin SA, Mehrabani D, Hosseini SV, Erfani N, et al. PD-1 gene polymorphisms in Iranian patients with colorectal cancer. Lab Medicine 2013;44:241-4.

26. Dong W, Gong M, Shi Z, Xiao J, Zhang J, Peng J. Programmed Cell Death-1 Polymorphisms Decrease the Cancer Risk: A Meta-Analysis Involving Twelve Case-Control Studies. PLoS One 2016;11:e0152448.
27. Tang W, Wang Y, Jiang H, Liu P, Liu C, Gu H, et al. Programmed death-1 (PD-1) rs2227981 C> T polymorphism is associated with cancer susceptibility: a meta-analysis. Int J Clin Exp Med 2015;8:22278-85.
28. Mamat U, Arkinjan M. Association of programmed death-1 gene polymorphism rs2227981 with tumor: evidence from a meta analysis. Int J Clin Exp Med 2015;8:13282-8.
29. Yin L, Guo H, Zhao L, Wang J. The programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with non-small cell lung cancer risk in a Chinese Han population. Int J Clin Exp Med 2014;7:5832-6.
30. Ma Y, Liu X, Zhu J, Li W, Guo L, Han X, et al. Polymorphisms of co-inhibitory molecules (CTLA-4/PD-1/PD-L1) and the risk of non-small cell lung cancer in a Chinese population. Int J Clin Exp Med 2015;8:16585-91.