Original Article
Programmed death-1 (*PD-1*) rs2227981 C > T polymorphism is associated with cancer susceptibility: a meta-analysis

Weifeng Tang[1*], Yafeng Wang[2*], Heping Jiang[3*], Pinghua Liu[4], Chao Liu[5], Haiyong Gu[5], Shuchen Chen[1], Mingqiang Kang[1]

[1]Department of Thoracic Surgery, The Union Clinical Medical College of Fujian Medical University, Fuzhou, Fujian Province, China; [2]Department of Cardiology, The People's Hospital of Xishuangbanna Dai Autonomous Prefecture, Jinghong, Yunnan Province, China; [3]Department of Emergency, Affiliated Jintan People's Hospital of Jiangsu University, Jintan, China; [4]Department of Anesthesiology, The People's Hospital of Xishuangbanna Dai Autonomous Prefecture, Jinghong, Yunnan Province, China; [5]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China. *Equal contributors.

Abstract: Several studies have focused on the correlation between the *programmed death-1* (*PD-1*) rs2227981 C > T polymorphism and the risk of cancer; however, the results of such studies remain conflicting. To address this gap, we performed this meta-analysis to identify the potential association. Search strategies were performed in PubMed and EMBASE using appropriate terms. In total, 2,977 cancer cases and 2,642 controls from seven publications were recruited in our study. According to the seven eligible publications, the odds ratios (ORs) and 95% confidence intervals (CIs) on the risk of cancer for the TT vs. CC and TT vs. CT+CC genotypes were 0.67 and 0.50-0.91 and 0.65 and 0.47-0.90, respectively. In a subgroup analysis by cancer type, *PD-1* rs2227981 C > T polymorphism was associated with a significantly decreased risk of breast cancer (OR, 0.82; 95% CI, 0.71-0.95; P = 0.009 for T vs. C and OR, 0.76; 95% CI, 0.63-0.92; P = 0.005 for TT+CT vs. CC) and of other cancer (OR, 0.58; 95% CI, 0.36-0.92; P = 0.004 for TT vs. CT+CC). In a subgroup analysis by ethnicity, a significant decreased cancer risk was identified among Asians (OR, 0.74; 95% CI, 0.63-0.86; P < 0.001 for T vs. C and OR, 0.71; 95% CI, 0.59-0.87; P = 0.001 for TT+CT vs. CC) and among Caucasians (OR, 0.66; 95% CI, 0.44-0.99; P = 0.047 for TT vs. CT+CC). These findings highlight the fact that the T allele of *PD-1* rs2227981 C > T polymorphism modestly decreases the susceptibility of cancer. Nevertheless, further large and well-designed studies are needed to enrich the evidence of this association.

Keywords: Polymorphism, programmed death-1, cancer risk

Introduction

It is estimated that about 14.1 million new cancer cases and 8.2 million cancer-associated deaths occurred in 2012 worldwide [1]. With new cases and mortality arising annually, cancer constitutes an enormous public health burden worldwide. These situations encourage researchers to explore the association of the latent environmental and genetic factors with the susceptibility of cancer. The aetiology of cancer is very elusive and has not been clarified thoroughly, although a number of investigations have focused on the function of the immune system [2, 3]. Immune-related genetic mutations may also affect the risk of cancer [4, 5].

Programmed death-1 (PD-1, also named CD279 or PDCD1), a co-inhibitory receptor that suppresses the activation of T lymphocytes and leads to peripheral immune tolerance, has been suggested to be involved in influencing the tumor cells to escape the host immune system after interaction with its two ligands, PD-1 ligand 1 (PD-L1) and PD-L2. PD-Ls are expressed in various malignancies [6-10]. In addition, up-regulation of PD-Ls in some cancers can contribute to tumor evasion and is associated with poor prognosis of malignancies
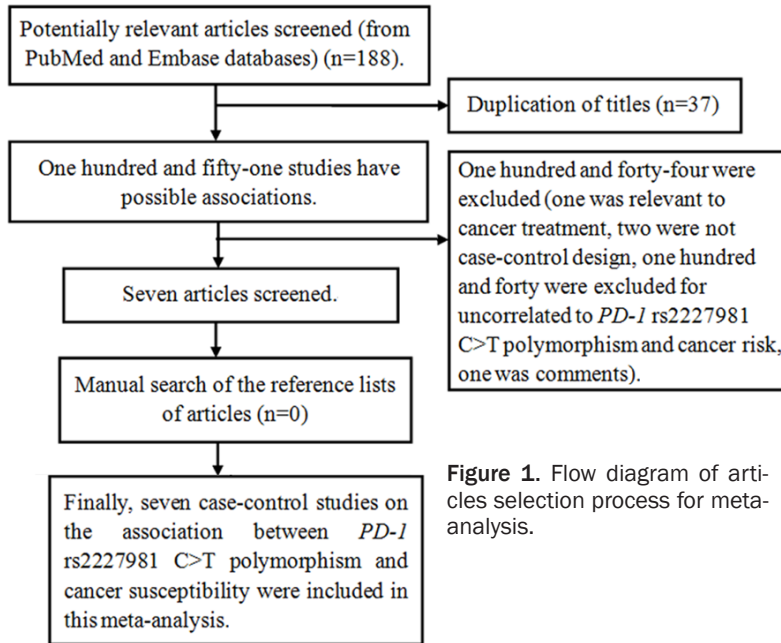
Figure 1. Flow diagram of articles selection process for meta-analysis.

Materials and methods

Search strategy

PubMed and EMBASE databases were used to search the potential papers which were published before March 4, 2015 without any language restriction. The following terms were used: 'polymorphism' or 'variant' or 'SNP' and 'programmed death-1', 'PD-1' or 'PDCD1' and 'cancer' or 'carcinoma' or 'malignance'.

Inclusion criteria and exclusion criteria

The major criteria were used to include eligible studies: (a) case-control studies; (b) studies that provided sufficient data to calculate crude odds ratios (ORs) and 95% confidence intervals (CIs) and (c) those assessing the correlation between the *PD-1* rs2227981 C > T polymorphism and the risk of cancer. The major excluded criteria were: (1) case reports, system reviews, editorials, letters, and comments; (2) not case-control study and (3) duplicated publications.

Data extraction

Two reviewers (W. Tang and Y. Wang) screened and extracted data independently. If there were any discrepancies, differences were adjudicated through discussions between all reviewers. The following information was extracted from every study: *PD-1* rs2227981 C > T polymorphism information, first author's surname, year of publication, country, ethnicity and sample size.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) in the controls for individual study was assessed using an internet-based HWE programme (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) and violation of HWE was defined by $P < 0.05$. Correlation between *PD-1* rs2227981 C > T polymorphism and cancer susceptibility was assessed using crude

[11-14]. Additionally, the function of regulatory T (Treg) cells also can be regulated through PD-1 pathway in cancer patients. Several recent investigations highlighted a correlation of PD-1 blockade with down-regulation of foxp3 expression by Treg cells in malignancy patients to correct immune escape [15-17].

The *PD-1* gene lies in chromosome 2q37.3, encoding a 55 KDa type I transmembrane glycoprotein. Several researchers have reported single nucleotide polymorphisms (SNPs) within the *PD-1* gene, such as rs36084323 A > G (PD-1.1), rs11568821 G > A (PD-1.3), rs2227981 C > T (PD-1.5), rs10204525 A > G (PD-1.6), rs7421861 T > C (PD-1.7), and rs2227982 C > T (PD-1.9) et al. Among these polymorphisms of the *PD-1* gene, one of the most widely studied SNPs is rs2227981 C > T polymorphism with minor allele frequency (MAF) > 0.05. This very SNP is located in exon 5 and does not affect the final amino acid residue (a synonymous mutation; Ala to Ala). To date, a few studies have explored the correlation of *PD-1* rs2227981 C > T polymorphism with caner susceptibility [18-24]; however, the results were conflicting. Thus, we performed this meta-analysis by pooling data from eligible case-control studies to further clarify the role of the *PD-1* rs2227981 C > T polymorphism in cancer.

Table 1. Characteristics of the individual studies included in the meta-analysis

Study	Year	Country	Ethnicity	Cancer type	Case/control	Genotype method
Yin *et al.* [18]	2014	China	Asians	Lung cancer	324/330	PCR-RFLP
Savabkar *et al.* [23]	2013	Iran	Caucasians	Gastric cancer	122/166	PCR-RFLP
Mojtahedi *et al.* [22]	2012	Iran	Caucasians	Colorectal cancer	200/200	PCR-RFLP
Haghshenas *et al.* [20]%	2011	Iran	Caucasians	Breast cancer	443/328	PCR-RFLP
Hua *et al.* [21]	2011	China	Asians	Breast cancer	490/512	PCR-RFLP
Ivansson *et al.* [24]	2010	Sweden	Caucasians	Cervical cancer	1306/811	TaqMan
Dehaghani *et al.* [19]	2009	Iran	Caucasians	Gestational trophoblastic neoplasm	92/295	PCR-RFLP

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism.

Table 2. Distribution of *PD-1* rs2227981 C > T polymorphism genotype and allele

Study	Year	Case			Control			Case		Control		HWE
		CC	CT	TT	CC	CT	TT	T	C	T	C	
Yin *et al.* [18]	2014	198	106	20	181	105	44	146	502	193	467	No
Savabkar et al. [23]	2013	50	66	6	89	70	7	78	166	84	248	Yes
Mojtahedi *et al*. [22]	2012	59	109	32	75	89	36	173	227	161	239	Yes
Haghshenas *et al.* [20]	2011	194	191	50	137	145	46	291	579	237	419	Yes
Hua *et al*. [21]	2011	295	169	22	244	210	24	213	759	258	698	Yes
Ivansson *et al.* [24]	2010	471	603	226	257	375	178	1055	1545	731	889	Yes
Dehaghani *et al.* [19]	2009	42	37	13	118	56	121	63	121	298	292	No

ORs together with corresponding 95% CIs. The Q-statistic and I^2 statistical tests were harnessed to measure the heterogeneity among studies. If the value of $I^2 > 50\%$ or $P < 0.10$ suggests substantial heterogeneity, random-effects models using DerSimonian-Laird method were used [25], otherwise, fixed-effects models using Mantel-Haenszel methods was performed [26]. The Begg's funnel plot [27] and Egger's linear regression [28] were used to assessed the potential publication bias. One-way sensitivity analysis was harnessed to assess the stability of our results. All analyses were conducted by the Stata 12.0 statistical software (Stata Corp LP, College Station, TX, USA). A $P < 0.05$ (two-sided) was defined as a statistically significant difference.

Results

Study characteristics

According to the search keywords and subject terms from the databases of PubMed and EMBASE, one hundred and eighty-eight potential correlated publications were enrolled. Based on the included criteria, seven studies were identified [18-24] (**Figure 1**). Among them, two case-control studies deviated from HWE [18, 19]. Five case-control studies focused on

Caucasians [19, 20, 22-24], two focused on Asias [18, 21]. Of these articles, two investigated breast cancer [20, 21], the others investigated lung cancer [18], colorectal cancer [22], gastric cancer [23], gestational trophoblastic neoplasm [19] and cervical cancer [24]. Characteristics from each included study were listed in **Table 1**. The genotype numbers and *P* value of HWE for the eligible studies were summarized in **Table 2**.

Quantitative synthesis

A total of 2,977 cancer cases and 2,642 controls from seven eligible investigations were enrolled. Our findings highlighted the statistical evidence of association between *PD-1* rs2227981 C > T variants and a decreased risk of malignance in two genetic models: TT vs. CC (OR, 0.67; 95% CI, 0.50-0.91; *P* = 0.011) and TT vs. CT+CC (OR, 0.65; 95% CI, 0.47-0.90; *P* = 0.009) (**Table 3** and **Figure 2**). In a subgroup analysis by cancer type, *PD-1* rs2227981 C > T polymorphism was associated with a significantly decreased risk of breast cancer (OR, 0.82; 95% CI, 0.71-0.95; *P* = 0.009 for T vs. C and OR, 0.76; 95% CI, 0.63-0.92; *P* = 0.005 for TT+CT vs. CC) and of other cancer (OR, 0.58; 95% CI, 0.36-0.92; *P* = 0.004 for TT vs. CT+CC). In a subgroup analysis by ethnicity, a significant

Table 3. Meta-analysis of the *PD-1* rs2227981 C > T polymorphism and cancer risk

	No. of study	T vs. C			TT vs. CC			TT+CT vs. CC			TT vs. CT+CC		
		OR (95% CI)	P	P (Q-test)	OR (95% CI)	P	P (Q-test)	OR (95% CI)	P	P (Q-test)	OR (95% CI)	P	P (Q-test)
Total	7	0.84 (0.71-1.00)	0.050	0.001	**0.67 (0.50-0.91)**	**0.011**	0.032	0.91 (0.74-1.12)	0.381	0.007	**0.65 (0.47-0.90)**	**0.009**	0.008
Ethnicity													
Asians	2	**0.74 (0.63-0.86)**	**< 0.001**	0.647	0.56 (0.31-1.00)	0.051	0.154	**0.71 (0.59-0.87)**	**0.001**	0.510	0.61 (0.30-1.27)	0.188	0.073
Caucasians	5	0.90 (0.71-1.14)	0.367	0.001	0.73 (0.49-1.07)	0.101	0.032	1.03 (0.79-1.34)	0.843	0.015	**0.66 (0.44-0.99)**	**0.047**	0.009
Cancer type													
Breast cancer	2	**0.82 (0.71-0.95)**	**0.009**	0.301	0.76 (0.53-1.10)	0.147	0.975	**0.76 (0.63-0.92)**	**0.005**	0.159	0.83 (0.59-1.17)	0.292	0.750
Other cancer	5	0.85 (0.66-1.11)	0.238	< 0.001	0.64 (0.41-1.01)	0.056	0.010	1.00 (0.75-1.33)	0.994	0.009	**0.58 (0.36-0.92)**	**0.022**	0.003
HWE													
Yes	5	0.93 (0.79-1.10)	0.400	0.019	**0.77 (0.63-0.93)**	**0.006**	0.446	0.98 (0.74-1.29)	0.871	0.002	**0.79 (0.66-0.94)**	**0.007**	0.905
No	2	**0.61 (0.45-0.84)**	**0.002**	0.138	**0.36 (0.24-0.56)**	**< 0.001**	0.476	0.78 (0.60-1.01)	0.060	0.927	**0.32 (0.21-0.49)**	**< 0.001**	0.165

HWE: Hardy-Weinberg equilibrium. Bold values are statistically significant (*P* < 0.05).
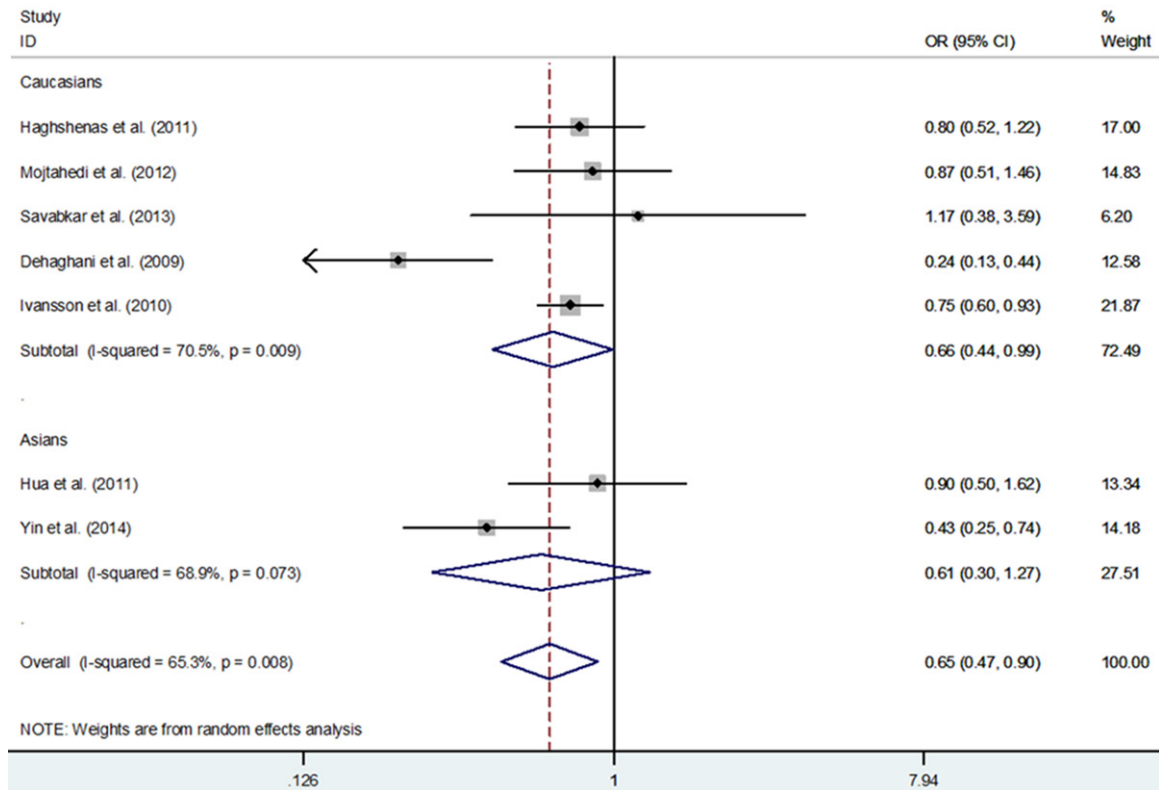
Figure 2. Meta-analysis with a fixed-effects model for the association between *PD-1* rs2227981 C > T polymorphism and cancer risk (TT vs. CT+CC genetic model).
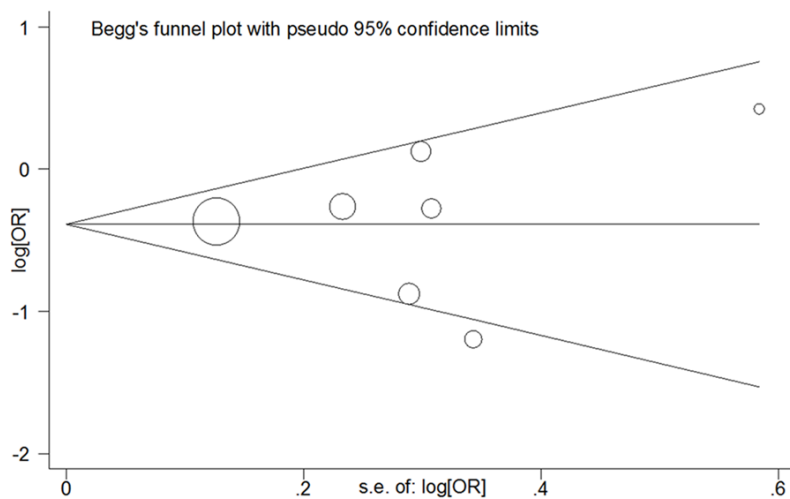

Figure 3. Begg's funnel plot of meta-analysis of the association between *PD-1* rs2227981 C > T polymorphism and the risk of cancer (TT vs. CC genetic model).

Publication bias

Begg's funnel plots and Egger's linear regression tests were harnessed to assess the publication bias (**Figure 3**). Significant publication bias was found in some genetic models (T vs. C: Begg's test *P* = 0.764, Egger's test *P* = 0.828; TT vs. CC: Begg's test *P* = 1.000, Egger's test *P* = 0.969; TT+CT vs. CC: Begg's test *P* = 0.133, Egger's test *P* = 0.148; TT vs. CT+CC: Begg's test *P* = 0.548, Egger's test *P* = 0.624).

decreased cancer risk was identified among Asians (OR, 0.74; 95% CI, 0.63-0.86; *P* < 0.001 for T vs. C and OR, 0.71; 95% CI, 0.59-0.87; *P* = 0.001 for TT+CT vs. CC) and among Caucasians (OR, 0.66; 95% CI, 0.44-0.99; *P* = 0.047 for TT vs. CT+CC).

Sensitivity analyses

Sensitivity analysis was conducted to assess the influence of anyone study on the pooled ORs and CIs by omitting an individual study in turn. Our findings showed that these results
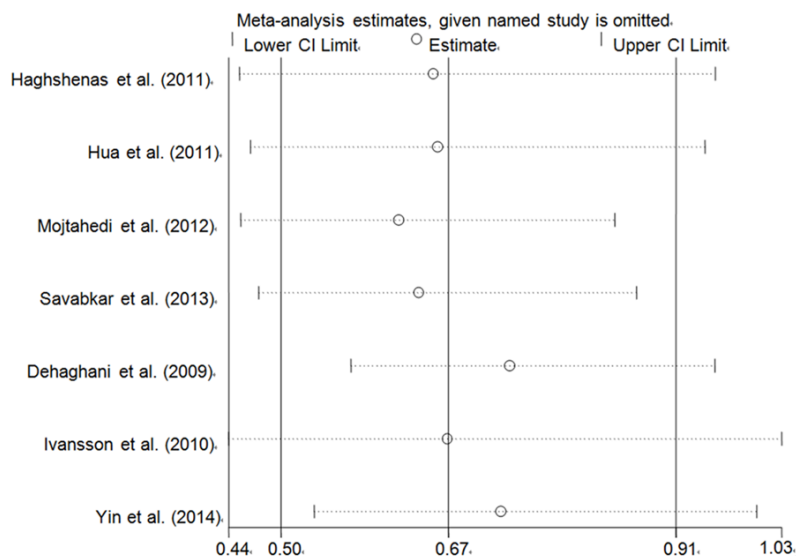
Figure 4. Sensitivity analysis of the influence of TT vs. CC compare genetic model (random-effects estimates for *PD-1* rs2227981 C > T polymorphism).

were robust and reliable (**Figure 4**) (data not shown).

Heterogeneity

As shown in **Table 3**, heterogeneity across the studies was significant in the current study. Thus, we assessed the sources of heterogeneity by race, the origin of cancer cells and HWE (**Table 3**). The findings showed that Caucasians and other cancer may contribute to the major sources of heterogeneity.

Discussion

In total, 2,977 cancer cases and 2,642 controls from seven eligible publications were recruited to investigate the correlation between the *PD-1* rs2227981 C > T polymorphism and the risk of cancer.

According to the results, the *PD-1* rs2227981 C > T polymorphism was suggested to be associated with a significantly decreased risk of cancer. The TT homozygote carriers suggested lower cancer incident susceptibility in comparison with the CC and CC+CT genotype carriers. The crude ORs and 95% CIs were 0.67 and 0.50-0.91, and 0.65 and 0.47-0.90, respectively. Although no statistical correlation between the *PD-1* rs2227981 C > T polymorphism and the risk of cancer was identified, when the genetic comparisons were carried out

between the TT+CT and CC homozygotes or between the T and C alleles, there remained a latent effect from the TT+CT genotypes or the T allele on the susceptibility of cancer. The crude ORs and 95% CIs were 0.91 and 0.74-1.12, respectively, for the TT+CT versus the CC homozygotes and 0.84 and 0.71-1.00, respectively, for the T versus C alleles.

The findings of this pooled study were supported by some investigations. In a previous study in China conducted by Hua *et al* [21], compared with the C allele, the T allele was a protective factor of breast cancer. Our findings were also supported by a Sweden study [24]. In this study (1, 306 cervical cancer cases and 811 controls), the TT homozygote reduced the risk of cervical cancer significantly. The ORs and 95% CIs were 0.69 and 0.54-0.89, respectively, for TT vs. CC and 0.75 and 0.60-0.93, respectively, for TT vs. CC+CT. The majority of our findings demonstrated the protective effect from the T allele on the susceptibility of cancer. In the future, further investigations based on a larger population and detailed gene-environment data are needed to be undertaken to confirm or refute our findings.

In the current study, two studies deviated from the HWE in controls, which showed the presence of population stratification and/or genotyping errors [18, 19]. When we omitted these two studies, the correlation between *PD-1* rs2227981 C > T polymorphism and cancer risk was also significant with respect to the two genetic models (OR, 0.77; 95% CI, 0.63-0.93; *P* = 0.006 for TT vs. CC and OR, 0.79; 95% CI, 0.66-0.94; *P* = 0.007 for TT vs. CT+CC; **Table 3**), attesting the robustness of our findings.

Several limitations of our study should be addressed when interpreting these findings. Due to the limited number of publications recruited in this pooled study, these findings should be interpreted with very caution. In addition, there were only two case-control studies

conducted in Asians, which may generate a fluctuated assessment or restrict the statistical power to detect a real influence. Moreover, in this meta-analysis, large heterogeneities across the studies included in the current analysis should also be taken into consideration. Finally, in this study, we only focused on *PD-1* rs2227981 C > T polymorphism, and did not ponder other *PD-1* polymorphisms or risk genes. However, our study also had several merits. First, to date, this is the first meta-analysis detecting the association of *PD-1* rs2227981 C > T polymorphism with the risk of cancer. The results demonstrated that this polymorphism was associated with the decreased risk of cancer. Second, although the large heterogeneities were identified in our study, the results of sensitivity analysis attesting the robustness of our findings.

In conclusion, our findings suggest that the T allele modestly decrease the susceptibility of cancer. Nevertheless, for practical reasons, further evidence from epidemiological studies across different populations incorporating with the functional assessments is required in order to confirm or refute the findings of this study.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Mingqiang Kang and Shuchen Chen, Department of Thoracic Surgery, The Union Clinical Medical College of Fujian Medical University, Fuzhou 350001, China. E-mail: Mingqiang_Kang@126.com (MQK); cscdoctor@163.com (SCC)

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Vendrame E, Martinez-Maza O. Assessment of pre-diagnosis biomarkers of immune activation and inflammation: Insights on the etiology of lymphoma. J Proteome Res 2011; 10: 113-119.

[3] de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 2006; 6: 24-37.

[4] Clifford RJ, Zhang J, Meerzaman DM, Lyu MS, Hu Y, Cultraro CM, Finney RP, Kelley JM, Efroni S, Greenblum SI, Nguyen CV, Rowe WL, Sharma S, Wu G, Yan C, Zhang H, Chung YH, Kim JA, Park NH, Song IH, Buetow KH. Genetic variations at loci involved in the immune response are risk factors for hepatocellular carcinoma. Hepatology 2010; 52: 2034-2043.

[5] Brenner AV, Neta G, Sturgis EM, Pfeiffer RM, Hutchinson A, Yeager M, Xu L, Zhou C, Wheeler W, Tucker MA, Chanock SJ, Sigurdson AJ. Common single nucleotide polymorphisms in genes related to immune function and risk of papillary thyroid cancer. PLoS One 2013; 8: e57243.

[6] Zou W, Chen L. Inhibitory b7-family molecules in the tumour microenvironment. Nat Rev Immunol 2008; 8: 467-477.

[7] Gao Q, Wang XY, Qiu SJ, Yamato I, Sho M, Nakajima Y, Zhou J, Li BZ, Shi YH, Xiao YS, Xu Y, Fan J. Overexpression of pd-l1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res 2009; 15: 971-979.

[8] Karim R, Jordanova ES, Piersma SJ, Kenter GG, Chen L, Boer JM, Melief CJ, van der Burg SH. Tumor-expressed b7-h1 and b7-dc in relation to pd-1+ t-cell infiltration and survival of patients with cervical carcinoma. Clin Cancer Res 2009; 15: 6341-6347.

[9] Hua D, Sun J, Mao Y, Chen LJ, Wu YY, Zhang XG. B7-h1 expression is associated with expansion of regulatory t cells in colorectal carcinoma. World J Gastroenterol 2012; 18: 971-978.

[10] Ohigashi Y, Sho M, Yamada Y, Tsurui Y, Hamada K, Ikeda N, Mizuno T, Yoriki R, Kashizuka H, Yane K, Tsushima F, Otsuki N, Yagita H, Azuma M, Nakajima Y. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res 2005; 11: 2947-2953.

[11] Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, Higuchi T, Yagi H,

Takakura K, Minato N, Honjo T, Fujii S. Programmed cell death 1 ligand 1 and tumor-infiltrating cd8+t lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A 2007; 104: 3360-3365.

[12] Chen YB, Mu CY, Huang JA. Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: A 5-year-follow-up study. Tumori 2012; 98: 751-755.

[13] Zheng Z, Bu Z, Liu X, Zhang L, Li Z, Wu A, Wu X, Cheng X, Xing X, Du H, Wang X, Hu Y, Ji J. Level of circulating pd-l1 expression in patients with advanced gastric cancer and its clinical implications. Chin J Cancer Res 2014; 26: 104-111.

[14] Zeng Z, Shi F, Zhou L, Zhang MN, Chen Y, Chang XJ, Lu YY, Bai WL, Qu JH, Wang CP, Wang H, Lou M, Wang FS, Lv JY, Yang YP. Upregulation of circulating pd-l1/pd-1 is associated with poor post-cryoablation prognosis in patients with hbv-related hepatocellular carcinoma. PLoS One 2011; 6: e23621.

[15] Wang W, Lau R, Yu D, Zhu W, Korman A, Weber J. Pd1 blockade reverses the suppression of melanoma antigen-specific ctl by cd4+ cd25(hi) regulatory t cells. Int Immunol 2009; 21: 1065-1077.

[16] Guo Z, Wang X, Cheng D, Xia Z, Luan M, Zhang S. Pd-1 blockade and ox40 triggering synergistically protects against tumor growth in a murine model of ovarian cancer. PLoS One 2014; 9: e89350.

[17] Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of pd-1 and ctla-4 combined with tumor vaccine effectively restores t-cell rejection function in tumors. Cancer Res 2013; 73: 3591-3603.

[18] 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: 5832-5836.

[19] Dehaghani AS, Kashef MA, Ghaemenia M, Sarraf Z, Khaghanzadeh N, Fattahi MJ, Ghaderi A. Pdcd1, ctla-4 and p53 gene polymorphism and susceptibility to gestational trophoblastic diseases. J Reproduct Med 2009; 54: 25-31.

[20] 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: 4205-4210.

[21] Hua Z, Li D, Xiang G, Xu F, Jie G, Fu Z, Jie Z, Da P, Li D. Pd-1 polymorphisms are associated with sporadic breast cancer in chinese han population of northeast china. Breast Cancer Res Treat 2011; 129: 195-201.

[22] 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: 229-232.

[23] 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: 178-182.

[24] Ivansson EL, Juko-Pecirep I, Gyllensten UB. Interaction of immunological genes on chromosome 2q33 and ifng in susceptibility to cervical cancer. Gynecol Oncol 2010; 116: 544-548.

[25] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177-188.

[26] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22: 719-748.

[27] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994; 50: 1088-1101.

[28] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634.