Cancer Epidemiology 58 (2019) 153–159

Contents lists available at ScienceDirect

Cancer Epidemiology

journal homepage: www.elsevier.com/locate/canep

Association of rs11801299 and rs1380576 polymorphisms at MDM4 with risk, clinicopathological features and prognosis in patients with retinoblastoma

Fenghua Yu[a], Zhongming Jiang[a], Aiping Song[b,*]

[a] Department of Ophthalmology, Central Hospital of Linyi, Yishui, Shandong, 276400, China
[b] Department of Ophthalmology, Shandong Provincial Qianfoshan Hospital, Jinan, Shandong, 250014, China

ARTICLE INFO

Keywords:
Retinoblastoma
MDM4
Polymorphism
Prognosis

ABSTRACT

Background: rs11801299 and rs1380576, two novel polymorphisms in *MDM4* gene, have been investigated in several different cancer types. However, the role of these two polymorphisms in retinoblastoma (RB) remains unclear.

Methods: A total of 126 patients with primary RB and 148 age-/gender-matched controls were included in this retrospective study. The frequency of rs11801299 and rs1380576 were determined between RB patients and controls. The association of these two polymorphisms with clinicopathological characteristics, prognosis were further evaluated.

Results: AA genotype at rs11801299 was significantly associated with an increased risk of developing RB (OR = 2.06, 95%CI 1.09–3.90). The possibility of developing RB was also significantly increased in individuals with A allele at rs11801299 (OR = 1.49, 95%CI 1.06–2.08). RB patients carrying AA genotype and A allele at rs11801299 were more likely to have tumor invasion and poor differentiation. As for rs1380576, a significantly lower risk of developing RB was observed in patients with G allele (CG + GG) compared with wild-type CC genotype (OR = 0.59, 95%CI 0.36–3.95). RB patients with GG genotype or G allele had a lower risk of developing highly aggressive cancer. Kaplan-Meier curves and log-rank results revealed that RB patients carrying AA genotype or A allele (AA + GA) at rs11801299 had significantly poorer prognosis. Multivariate COX analysis showed that the rs11801299 G allele was associated with decreased survival but was not an independent prognostic factor.

Conclusion: rs11801299 was significantly associated with RB risk, pathological differentiation, tumor aggressiveness and poor prognosis.

1. Introduction

Retinoblastoma (RB), the most frequent intraocular malignancy among children, can seriously affect an infant's vision and life [1]. RB is the first cancer for which a genetic etiology has been described. Originating from either germline or somatic mutations in the RB1 tumor suppressor gene, RB tumors can be divided into two types: heritable and non-heritable [2]. With rapid improvements in public and medical awareness and rigorous, innovative clinical treatments, RB is no longer considered a deadly childhood disease [3]. However, the prognosis varies widely around the world. In countries of low and middle income, mortality from retinoblastoma is approximately 70%. Because survival and the chance of saving the patient's vision depend on the severity of the disease at presentation, early diagnosis and accurate prediction of prognosis are vital to improve survival time. Thus, identifying a genetic biomarker of RB progression could promote a deeper understanding of RB pathogenesis and lead to the development of molecular prognostic indicators and therapeutic strategies [4–6].

In addition to RB gene, it has been widely acknowledged that p53 pathway has a crucial function in RB pathogenesis. p53 pathway is the master control system of cell cycle, genome stability and cell apoptosis. Maintenance of normal p53 levels is vital for cell life and tissue homeostasis, as well as for protection of genomic stability, which can be modulated by a negative feedback loop in which p53 transcriptionally activates MDM2 [7,8]. MDM2 (murine double-minute 2 homology) functions as a negative regulator in p53 pathway, and its activation could cause increased proteolytic degradation of p53 [9]. *MDM4* gene (also known as *MDMX* or *HDMX*), located in chromosome 1q32, is an

* Corresponding author.
E-mail address: shengzeiyou2@163.com (A. Song).

https://doi.org/10.1016/j.canep.2018.12.010
Received 26 August 2018; Received in revised form 28 October 2018; Accepted 17 December 2018
Available online 04 January 2019
1877-7821/

MDM2 homologue with high structural similarity which has been shown to promote the proteasome-mediated degradation of p53 and negatively regulate p53 pathway [10–14].

Recent data have suggested that *MDM4* gene polymorphisms are related with the risk of developing various cancers [15,16]. In 2012, de Oliveira Reis et al. reported that the frequency of MDM4 polymorphism rs116197192 G allele was significantly higher in RB patients and could increase the risk of developing RB, whereas MDM4 polymorphism rs4252668 was not associated with RB risk [17]. Recent studies have suggested a potential association of rs11801299, rs1380576 in *MDM4* gene with the development of multiple cancers, such as gastric cancer, breast cancer, and OSCC [18–20]. However, there are currently no reports about the association of these two polymorphisms with RB risk. Given that MDM4 could affect p53 activity and be involved in the DNA damage response and preventing cancer pathogenesis, we hypothesized that MDM4 polymorphisms might be susceptible genetic factors for RB development. Therefore, the purpose of the present study was to analyze the distribution of rs11801299 and rs1380576, further assess their association with clinicopathological characteristics and prognosis in RB patients.

2. Materials and methods

2.1. Participants

This retrospective study enrolled 126 RB patients who were recruited in Qianfoshan Hospital (Shandong, China) and Linyi Center Hospital (Shandong, China). All RB patients were examined by independent histopathologists. 148 gender-matched and ethnicity-matched normal controls (unrelated healthy individuals) were also included. All controls were adult individuals who underwent health examinations without any history of cancer during the same periods in same hospital. All aspects of the present study were approved by the Research Ethics Committee of Qianfoshan Hospital of Jinan and the Research Ethics Committee of Linyi Center Hospital, and all individuals and/or their parents gave informed consent to participate in this study.

2.2. Blood samples and medical data extraction

Approximately 5 ml of peripheral vein blood sample was collected from each participant into a tube containing EDTA. Clinical and laboratory information about all RB patients were extracted from medical records, including age at diagnosis, gender, family history of RB, laterality, tumor aggressiveness, tumor invasion, tumor aggressiveness, massive choroidal invasion, scleral invasion, optic nerve cut end invasion and lag time between diagnosis and treatment onset. Overall survival time was defined as the interval from diagnosis until death or the last follow-up. The survival time was defined as the time from surgery day until the date of RB-related death or the last follow-up.

2.3. MDM4 polymorphism analysis

Genomic DNA was extracted from blood samples using a Genomic DNA Kit (Axygen, USA) according to the manufacturers' protocol, and genomic DNA samples were stored at -20^{0}C until use. Two valid SNPs (rs11801299 and rs1380576) located in MDM4 gene were genotyped. Genotyping for these two SNPs was performed using custom TaqMan® SNP Genotyping Assays (ABI, CA, USA). Genotyping or allele analysis were carried out with the ABI Prism 7900 H T genetic detection system, which showed the results of allelic discrimination plot by automatic allele analysis. The vertical axis indicated a wild-type GG or CC homozygote, the horizontal axis indicated a mutant type homozygote of AA, GG, and the diagonal indicated a GA and CG heterozygote for rs11801299 or rs1380576, respectively.

2.4. Statistical analysis

Continuous data were compared between RB patients and normal controls using the *T* test, while categorical data were compared using chi-square tests. Association between genotypes and RB risk were estimated by odds ratios (ORs) using an unconditional logistic regression model. Survival probabilities were estimated using Kaplan–Meier analysis, and significant differences were analyzed using the log-rank test. Cox proportional hazards models were used to analyze the associations between genotypes with RB survival. Hazard ratios (HR) and 95% confidence intervals were estimated using multivariate models. The deviation from Hardy-Weinberg equilibrium was assessed using $\chi 2$ tests. All statistical analyses were performed in SPSS 18.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Participant characteristics

A total of 126 RB patients and 148 non-carriers controls were included. All participants were of Han ethnicity and from the same region in Shandong, China. No significant differences between RB patients and normal controls in gender were detected ($P = 0.47$). The clinical characteristics of RB patients were presented in Table 1.

3.2. Association of rs11801299 with RB risk

The distribution and statistical analyses of rs11801299 genotypes in RB patients and normal controls were summarized in Table 2. The value for the $\chi 2$ tests of HWE was 0.23 for the controls. Significant difference could be observed in the frequency of rs11801299 genotype between RB patients and controls. Compared with wild-type GG genotype at rs11801299, AA genotype was significantly associated with increased

Table 1
Clinical characteristics of 126 retinoblastoma patients.

Characteristics	RB patients (n, %)
Gender	
Male	56 (56.9)
Female	70 (43.1)
Age at diagnosis (Months)	
< 24	88 (70.8)
> 24	38 (29.2)
Differentiation	
Well or Moderate	60 (47.6)
Poor	66 (52.4)
Laterality	
Unilateral	96 (75.2)
Bilateral	30 (24.8)
Invasion	
Negative	54 (43.1)
Positive	72 (56.9)
Choroidal Invasion	
Minor(< 3 mm)	38 (57.6)
Massive (≥3 mm)	28 (42.4)
Scleral Invasion	
Yes	13 (10.3)
No	113 (89.7)
Optic Nerve Cut End Invasion	
Yes	11 (8.7)
No	115 (91.3)
Tumor aggression	
Low	54 (42.3)
High	72 (57.7)
Lag-time (Months)	
< 3	101 (78.8)
≥3	25 (21.2)

Invasion included choroidal invasion, scleral invasion or optic nerve invasion.

Table 2
Genotype distribution and allele frequencies of rs11801299 and rs1380576 at MDM4 gene in both RB patients and normal controls.

Genotype		RB Patients (n, %)	Controls (n, %)	HWE	OR	95%CI	*P* value
rs11801299				0.23			
Genotype	G/G	39 (30.9)	57 (38.5)		1	–	
	G/A	49 (38.9)	64 (43.2)		1.12	0.65-1.94	0.78
	A/A	**38 (30.2)**	**27 (18.3)**		**2.06**	**1.09-3.90**	**0.04**
	G/A + A/A	87 (69.1)	91 (61.5)		1.40	0.85-2.31	0.102
Allele	G	127 (50.4)	178 (60.1)		1	–	
	A	**125 (49.6)**	**118 (39.9)**		**1.49**	**1.06-2.08**	**0.03**
rs1380576				0.29			
Genotype	C/C	77 (61.1)	71 (47.9)		1	–	
	C/G	39 (30.9)	59 (39.9)		0.61	0.36-1.03	0.07
	G/G	10 (8.0)	18 (12.2)		0.51	0.22-1.19	0.15
	C/G + G/G	**49 (38.9)**	**77 (39.7)**		**0.59**	**0.36-0.95**	**0.04**
Allele	C	193 (76.6)	201 (67.9)		1	–	
	G	**59 (23.4)**	**95 (32.1)**		**0.65**	**0.44-0.95**	**0.03**

The significance of bold values means significantly statistical difference.

risk of developing RB (OR = 2.06, 95%CI 1.09–3.90). Similarly, compared with G allele, the possibility of developing RB was also significantly increased in individuals with A allele at rs11801299 (OR = 1.49, 95%CI 1.06–2.08). The estimated risk of developing RB in individuals carrying GA or AA genotype was 1.4-fold higher than those with wild-type GG, though this difference was not statistically significant (OR = 1.40, 95%CI 0.85–2.31). Besides, no significant differences in the frequencies of GG or GA genotypes were observed between RB patients and normal controls.

3.3. Association of rs1380576 with RB risk

As shown in Table 2, the analysis did not yield a significant deviation from HWE in control group (P = 0.29). A significantly decreased risk for RB was observed in subjects with CG or GG genotype under recessive model compared to wild-type CC genotype (OR = 0.59,95%CI 0.36–3.95). In addition, individuals with G allele at rs1380576 had an almost 35% decreased risk of developing RB compared with those with C allele (OR = 0.65, 95%CI 0.44-0.95). Besides, compared with CC genotype at rs1380576, no significant differences in the distributions of either CG or GG genotypes were observed between RB patients and normal controls.

3.4. Association of rs11801299 and rs1380576 with clinicopathological characteristics in RB patients

The association of rs11801299 and rs1380576 with clinicopathological features in RB patients were further evaluated. Table 3 showed the subgroup analysis stratified by gender, age at diagnosis, family history of RB, laterality, tumor invasion, tumor aggressiveness, massive choroidal invasion, scleral invasion, optic nerve cut end invasion, and lag time. RB patients carrying AA genotype and A allele at rs11801299 were more likely to have tumor invasion (OR = 2.95, 95%CI 1.13–7.67 and OR = 1.87, 95%CI 1.13–3.10, respectively). Additionally, RB patients carrying AA genotype and A allele at rs11801299 were also more likely to have poor tumor differentiation (OR = 2.77, 95%CI 1.09–7.14 and OR = 1.84, 95%CI 1.12–3.04, respectively). RB patients carrying A allele at rs11801299 also have higher risk for optic nerve cut end invasion than those with G allele (OR = 2.96, 95%CI 1.12–7.84; P = 0.03). As for rs1380576, we observed that RB patients with GG genotype or G allele were at a lower risk for high tumor aggressiveness compared to those with CC genotype or C allele (OR = 0.23, 95%CI 0.06-0.95 and OR = 0.46, 95%CI 0.25-0.83, respectively). Besides, no significant associations between rs11801299 or rs1380576 and other characteristics were observed.

3.5. Association of rs11801299 and rs1380576 with RB prognosis

All RB patients received enucleation therapy and a 50-month follow-up. Kaplan–Meier curves were built to evaluate the association of survival rate with rs11801299 and rs1380576. Kaplan-Meier curves and log-rank results revealed that RB patients carrying AA genotype had significantly worse survival rates than those with GG or GA (P < 0.01 for both, Fig. 1a). Consistently, RB patients with allele A (AA + GA) also had a much shorter survival time compared with individuals with GG (P < 0.01, Fig. 1b). However, a significant difference in survival rate was not detected among patients with different genotypes at rs1380576. RB patients carrying GG genotype at rs1380576 had a much longer survival time than those with CC and CG genotypes alone, though this difference was not statistically significant (Fig. 1c, P = 0.12, P = 0.15). Meanwhile, RB patients carrying G allele (CG + GG) did not exhibit any differences in overall survival compared to CC genotype (Fig. 1d, P = 0.13). In addition, the results of multivariate analysis of survival time using Cox proportional hazards model were presented in Table 4. Univariate Cox analysis showed that tumor pathological differentiation, tumor invasion, tumor aggression, lag time as well as rs11801299 were associated with survival time in RB patients. In multivariate Cox analysis, tumor invasion and lag time were two independent risk factors related to overall survival in RB patients. Neither rs11801299 nor rs1380576 polymorphism was associated independently with overall survival time.

4. Discussion

Since the two-hit hypothesis for tumor initiation was proposed, RB has been considered a prototypic model cancer [6]. Although the inactivation of RB1 tumor suppressor gene is sufficient to initiate the onset of this tumor, its development may be modified by numerous additional genetic mutations in RB patients [6,25]. In the present study, we have evaluated the association between rs11801299, rs1380576 and RB risk. rs11801299 mutation AA genotype and A allelec could increase RB risk and were significantly associated with tumor invasion and pathological differentiation as well as poor prognosis. Conversely, allele G (CC + CG) at rs1380576 decreased the risk of developing RB and of high tumor aggressiveness.

As a response to DNA damage, p53 signaling pathway could prevent cell proliferation through several mechanisms, including cell cycle arrest and apoptosis. The homeostasis of normal cells is related to the normal functioning of p53 pathway. If p53 pathway is impaired, homeostasis will be disrupted. Previous studies demonstrated that mouse double minute oncoprotein family proteins, including MDM2 and MDM4, play an important role in the maintenance of p53 pathway. First, MDM2 can regulate p53 levels by acting as an E3 ligase and

Table 3
Association of rs11801299 and rs1380576 with clinicopathological characteristics in RB patients.

Characteristics	rs11801299					rs1380576				
	Genotype			Allele		Genotype			Allele	
	G/G	G/A	A/A	G	A	C/C	C/G	G/G	C	G
Gender										
Female	20	26	24	66	74	42	22	6	106	34
Male	19	23	14	61	51	35	17	4	87	25
OR (95%)	1.0	1.07	1.63	1.0	1.34	1.0	0.93	0.80	1.0	0.90
	(-)	(0.46-2.49)	(0.66-4.05)	(-)	(0.82-2.21)	(-)	(0.43-2.05)	(0.21-3.06)	(-)	(0.50-1.61)
P value	–	0.86	0.36	–	0.26	–	0.85	0.74	–	0.77
Age at diagnosis										
< 24 months	26	34	28	86	90	53	28	7	134	42
> 24 months	13	15	10	41	35	24	11	3	59	17
OR (95%)	1.0	1.13	1.40	1.0	1.23	1.0	0.87	0.95	1.0	0.92
	(-)	(0.46-2.79)	(0.52-3.74)	(-)	(0.72-2.10)	(-)	(0.37-2.03)	(0.23-3.98)	(-)	(0.48-1.75)
P value	–	0.82	0.62	–	0.50	–	0.83	0.94	–	0.87
Differentiation										
Well/Moderately	23	24	13	70	50	40	15	5	95	25
Poorly	16	25	25	57	75	37	24	5	98	34
OR (95%)	1.0	1.49	**2.77**	1.0	**1.84**	1.0	1.73	108	1.0	1.32
	(-)	(0.64-3.57)	**(1.09-7.14)**	(-)	**(1.12-3.04)**	(-)	(0.79-3.79)	(0.29-4.04)	(-)	(0.73-2.38)
P value	–	0.40	**0.04**		**0.02**	–	0.24	0.91		0.38
Laterality										
Unilateral	28	38	30	94	98	59	29	8	147	45
Bilateral	11	11	8	33	27	18	10	2	36	14
OR (95%)	1.0	2.12	1.05	1.0	1.48	1.0	1.13	0.82	1.0	1.45
	(-)	(0.88-5.15)	(0.20-5.42)	(-)	(0.75-2.94)	(-)	(0.46-2.76)	(0.16-4.21)	(-)	(0.74-2.86)
P value	–	0.10	0.62		0.27	–	0.82	0.81		0.28
Invasion										
Negative	20	24	10	64	44	33	16	5	82	26
Positive	19	25	28	63	81	44	23	5	111	33
OR (95%)	1.0	1.10	**2.95**	1.0	**1.87**	1.0	1.08	0.75	1.0	0.93
	(-)	(0.47-2.54)	**(1.13-7.67)**	(-)	**(1.13-3.10)**	(-)	(0.49-2.36)	(0.20-2.81)	(-)	(0.52-1.69)
P value	–	0.86	**0.04**		**0.02**	–	0.85	0.74		0.88
Choroidal Invasion										
Minor	13	13	12	39	37	23	12	3	58	18
Massive	7	13	8	27	29	16	9	3	41	15
OR (95%)	1.0	1.99	1.27	1.0	1.14	1.0	1.02	1.02	1.0	1.02
	(-)	(0.58-6.95)	(0.33-4.84)	(-)	(0.56-2.33)	(-)	(0.39-3.09)	(0.15-6.86)	(-)	(0.44-2.35)
P value	–	0.36	0.64	–	0.86	–	1.00	1.00	–	1.00
Scleral Invasion										
No	36	44	33	116	110	70	35	8	175	51
Yes	3	5	5	11	15	7	4	2	18	8
OR (95%)	1.0	1.36	1.82	1.0	1.44	1.0	0.46	2.19	1.0	1.53
	(-)	(0.31-6.10)	(0.40-8.21)	(-)	(0.63-3.27)	(-)	(0.07-2.95)	(0.34-14.1)	(-)	(0.63-3.71)
P value	–	0.89	0.48	–	0.41	–	0.59	0.59	–	0.34
Optic Nerve Cut End Invasion										
No	38	45	32	121	109	73	34	8	180	50
Yes	1	4	6	6	16	4	5	2	13	9
OR (95%)	1.0	3.38	7.13	1.0	**2.96**	1.0	2.68	4.56	1.0	2.49
	(-)	(0.36-31.5)	(0.82-62.3)	(-)	**(1.12-7.84)**	(-)	(0.68-10.6)	(0.72-28.9)	(-)	(1.01-6.17)
P value	–	0.38	0.06	–	**0.03**	–	0.16	0.14	–	0.06
Aggression										
Low	22	29	13	70	55	27	20	7	74	34
High	17	20	25	57	70	50	19	3	119	25
OR (95%)	1.0	1.03	2.49	1.0	1.56	1.0	0.51	**0.23**	1.0	**0.46**
	(-)	(0.44-2.41)	(0.99-6.26)	(-)	(0.95-2.57)	(-)	(0.23-1.12)	**(0.06-0.95)**	(-)	**(0.25-0.83)**
P value	–	0.87	0.07		0.08	–	0.11	**0.04**		0.01
Lag-time										
< 3 months	32	38	31	102	100	60	34	7	154	48
> 3 months	7	11	7	25	25	17	5	3	39	11
OR (95%)	1.0	0.76	0.96	1.0	0.98	1.0	0.52	1.51	1.0	0.91
	(-)	(0.26-2.18)	(0.30-3.08)	(-)	(0.53-1.82)	(-)	(0.18-1.53)	(0.35-6.48)	(-)	(0.43-1.90)
P value	–	0.79	1.00	–	1.00	–	0.32	0.69	–	0.85

The significance of bold values means significantly statistical difference.

interacting with the terminal transaction domain of TP53, eventually attenuating the effects of excess p53 [26]. MDM4 was described as an MDM2 homologue with high similarity at the primary structural level [27]. However, unlike MDM2, MDM4 has no appreciable ubiquitin ligase activity. MDM4 may interact with MDM2 through RING domains to enhance the ability of MDM2 to regulate p53. Meanwhile, genetic studies revealed that MDM4 contributes primarily to inhibition of p53-mediated transcriptional transactivation, whereas MDM2 contributes primarily to p53 degradation [28]. In addition to affecting p53, some studies also suggested a p53-independent function of MDM4 in development. Studies in transgenic mice indicated that MDM4 upregulation promotes spontaneous tumor development and accelerates

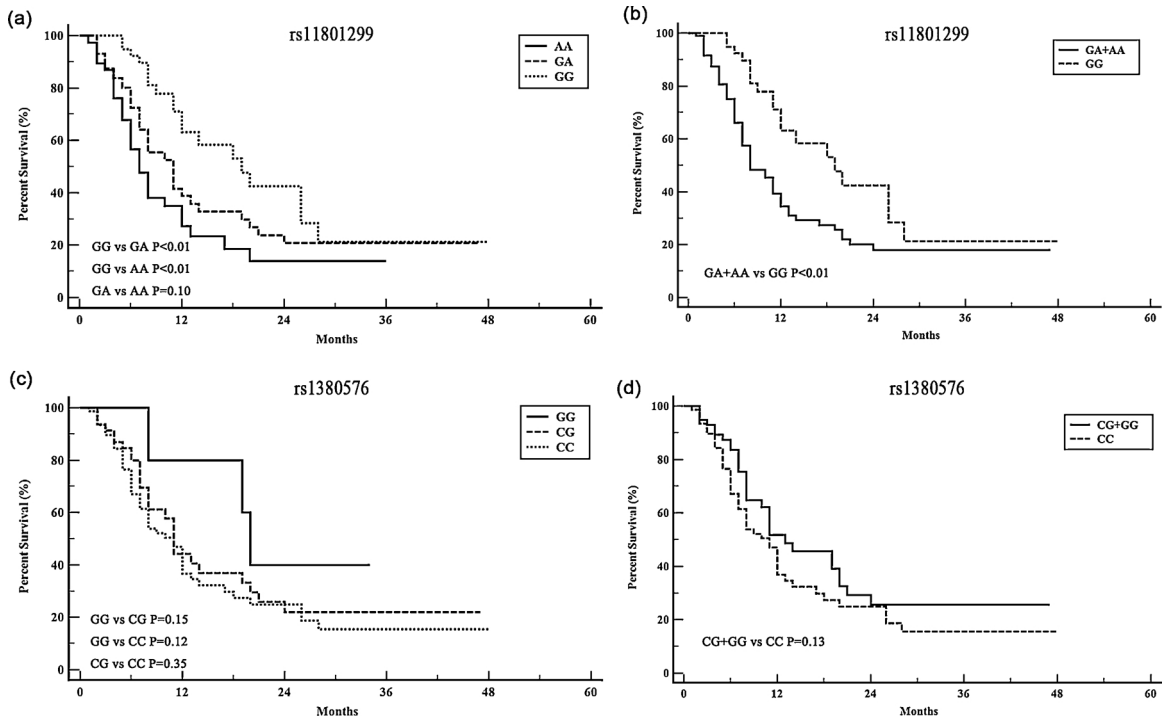

Fig. 1. Kaplan-Meier survival curves of RB patients with different MDM4 polymorphisms, rs11801299 (a, b) or rs1380576 (c, d). The MDM4 polymorphism rs11801299 (a, b), but not rs1380576 (c, d), was correlated with overall survival in RB patients.

carcinogenesis [29]. Amplification and overexpression of MDM4 has been observed in retinoblastomas in comparison to fetal retina. Functional experiments have shown that MDM4 promotes tumorigenesis in mice lacking Rb1/Rbl1 and treatment of RB cell line xenografts with the small molecule nutlin-3 which targets p53-MDM2/4 interaction could reduce tumor growth [30]. Subconjunctival delivery of nutlin-3 in preclinical models of RB demonstrated some efficacy in mediating p53-dependent cell death in RB [31]. Tp53 gene alteration is the most common molecular event driving several different types of cancer, and developing molecules to block p53-MDM2/MDM4 interactions is considered a promising strategy to combat cancers that contain inactive wild-type p53.

Until now, accumulated evidence has supported an association between MDM2, MDM4 gene polymorphisms and RB development, tumor features, and prognosis. For example, our group has shown that AG/GG genotype at MDM2 rs937283 significantly increases the risk of RB development, and RB patients with allele G at rs937283 were more susceptible to invasion, high tumor aggression, and poor prognosis [32]. The most widely studied MDM2 rs2279744 has also been linked to RB development in the Chinese population [33]. As for MDM4, previous studies have revealed that G allele of MDM4 rs116197192 is significantly higher in RB patients, suggesting an association with increased risk of RB development [17]. The other two most-studied MDM4 polymorphisms are rs11801299 and rs1380576. Previous studies have described the role of these two polymorphisms in different cancer types, including gastric cancer, breast cancer and others [17,18,20]. However, these studies yielded different results. Recently, Wang et al. reported that GG variant allele of MDM4 rs1380576 may reduce the risk of gastric cancer in an Eastern Chinese population [11]. Hashemi et al. also found that C > G polymorphism of MDM4 rs1380576 was a protective factor for BC risk in a Southeast Iranian population [18]. In contrast, Sun et al. observed that rs1380576 was associated with a higher risk of prostate cancer and a higher Gleason score, demonstrating that rs1380576 is related to more aggressive prostate cancers in American Caucasian population [34]. Consistent with these results, the present study also revealed that G allele (GG + CG) at rs1380576 could decrease the risk of developing RB. Of course, different tumor types may contribute to the differences observed in the association between rs1380576 and tumor risk, but this discrepancy may be also attributed to the different ethnicities studied. Further, the

Table 4
Multivariate Cox proportional hazard analysis of the prognostic factors for the overall survival rates of RB patients.

Factors	Categories	HR (95%CI)	
		Univariate	Multivariate
Age	> 24 m/ < 24 m	0.76 (0.49-2.49)	0.82 (0.58-2.35)
Gender	Male/Female	0.97 (0.67-2.21)	1.29 (0.88-2.91)
Pathological Grade	Poorly/Well + Moderately	**2.98 (1.45-4.63)**	2.30 (0.96-5.19)
Laterality	Unilateral/Bilateral	0.64 (0.34-2.03)	0.68 (0.45-1.07)
Tumor aggression	Low/High	**0.45 (0.28-0.89)**	0.51 (0.32-1.16)
Lag-time	> 3 m/ < 3 m	**3.70 (2.04-6.33)**	**1.98 (1.64-4.09)**
Invasion	Yes/No	**3.29 (1.70-4.69)**	**2.47 (1.58-6.29)**
rs11801299	AA + GA/GG	**1.97 (1.23-3.76)**	1.88 (0.93-3.08)
rs1380576	GG + GA/CC	0.83 (0.46-2.02)	0.67 (0.34-1.85)

The significance of bold values means significantly statistical difference.
HR: Hazard Ratio; 95% CI: 95% Confidence Interval; m, months; Invasion included choroidal invasion, scleral invasion or optic nerve invasion.

present study also evaluated the association between this polymorphism and tumor features and prognosis. Although no significant effect of rs1380576 on prognosis was observed, the data showed that GG genotype and G allele of rs1380576 were correlated with tumor aggressiveness in RB patients. Concerning MDM4 rs11801299, our results agree with those of Wang et al., who found that GA genotype increased the risk of developing gastric cancer in an Eastern Chinese population compared to GG genotype [20]. Similarly, we observed that AA genotype significantly increased the risk of developing RB. However, Hashemi et al. did not find any association between rs11801299 and breast cancer risk in a Southeast Iranian population [18]. Our results showed that rs11801299 polymorphism is related to tumor invasion, poor pathological differentiation, and poor prognosis. Recently, one interesting report examined whether rs11801299 polymorphism is associated with HPV16 status in squamous cell carcinoma of oropharynx [19]. Lu et al. reported that AG or AA genotypes at rs11801299 were more common in squamous cell carcinoma of oropharynx patients with HPV16-positive (37.6%) than those with HPV16-negative (25.6%; $P = 0.018$) [19].

rs11801299 (NC_000001.10:g.204529084 G > A) was located in the 3′-untranslated region (3′-UTR), whereas the other, rs1380576 (NC_000001.10:g.204488278 G > C) was in the first intron of MDM4 gene. The 3′-UTR and intron 1 of *MDM4* gene play vital role in gene-regulatory functions, affecting gene expression and tumor susceptibility through regulation of the mRNA stability and translational efficiency [21–23]. Hence, MDM4 expression could be altered by these functional genetic mutation in *MDM4* such as rs11801299 and rs1380576. For example, the previous study have conducted a luciferase reporter experiment to determine the effect of rs11801299 on MDM4 expression, and demonstrated that the rs11801299 variant A allele was associated with significantly lower luciferase activity compared with G allele [24]. Therefore, the A variant allele at rs11801299 might be biologically functional to reduce MDM4 expression in cancer cell.

Of course, there were several limitations in the present study. First, the sample size is relatively small. A small sample size may produce a small number of individuals carrying some homozygous genotype, especially GG genotype at rs1380576, which could limit the accuracy and reliability of these results. Based on the survival curve, patients with GG genotype of rs1380576 had better prognoses, but this difference was not statistically significant due to smaller sample size. Second, we did not elucidate the mechanism for how these polymorphisms affect RB risk and prognosis. We speculate that the effects may result from these two polymorphisms decreasing MDM4 production and thus further impairing the normal regulation of p53 pathway, but this hypothesis regarding the role of these two polymorphisms in MDM4 expression need to be confirmed. Third, the power of these two polymorphisms as major predicting factors of RB development or prognosis in clinical practice was relatively low. For example, the multiple COX analysis revealed that neither rs11801299 nor rs1380576 was an independent prognostic biomarker in RB patients. Thus, this study will need to be replicated in other, larger independent cohorts including different ethnicities.

In conclusion, our genetic assessment is the first study to evaluate the association of rs11801299 and rs1380576 with RB. The results showed that both AA genotype and A allele at rs11801299 were associated with an increased risk of developing RB as well as tumor invasion and poor pathological differentiation. Further, G allele at rs1380576 reduced the risk of developing RB and was associated with low tumor aggressiveness. In addition, G allele at rs11801299 reflected poor prognoses of RB patients.

Conflict of interest

All authors have declared no conflict of interest.

Authorship contribution statement

Data collect: Fenghua Yu, Zhongming Jiang, Aiping Song
Formal analysis: Fenghua Yu
Methodology: Fenghua Yu, Zhongming Jiang1, Aiping Song2
Statistical Software: Fenghua Yu, Zhongming Jiang
Supervision: Aiping Song
Writing – original draft: Fenghua Yu, Zhongming Jiang, Aiping Song
Writing – review & editing: Aiping Song

References

[1] H. Dimaras, K. Kimani, E.A. Dimba, P. Gronsdahl, A. White, H.S. Chan, B.L. Gallie, Retinoblastoma, Lancet 379 (9824) (2012) 1436–1446.
[2] I. Aerts, L. Lumbroso-Le Rouic, M. Gauthier-Villars, H. Brisse, F. Doz, L. Desjardins, Retinoblastoma, Orphanet J. Rare Dis. 1 (2006) 31.
[3] P. Lin, J.M. O'Brien, Frontiers in the management of retinoblastoma, Am. J. Ophthalmol. 148 (2) (2009) 192–198.
[4] S.S. Liu, Y.S. Wang, Y.F. Sun, L.X. Miao, J. Wang, Y.S. Li, H.Y. Liu, Q.L. Liu, Plasma microRNA-320, microRNA-let-7e and microRNA-21 as novel potential biomarkers for the detection of retinoblastoma, Biomed. Rep. 2 (3) (2014) 424–428.
[5] S. Su, J. Gao, T. Wang, J. Wang, H. Li, Z. Wang, Long non-coding RNA BANCR regulates growth and metastasis and is associated with poor prognosis in retinoblastoma, Tumour Biol. 36 (9) (2015) 7205–7211.
[6] I.N. Carvalho, A.H. Reis, P.H. Cabello, F.R. Vargas, Polymorphisms of CDKN1A gene and risk of retinoblastoma, Carcinogenesis 34 (12) (2013) 2774–2777.
[7] E. Eskandari-Nasab, M. Hashemi, S. Amininia, M. Ebrahimi, M. Rezaei, S.M. Hashemi, Effect of TP53 16-bp and beta-TrCP 9-bp INS/DEL polymorphisms in relation to risk of breast cancer, Gene 568 (2) (2015) 181–185.
[8] J.D. Oliner, J.A. Pietenpol, S. Thiagalingam, J. Gyuris, K.W. Kinzler, B. Vogelstein, Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53, Nature 362 (6423) (1993) 857–860.
[9] M.E. Perry, The regulation of the p53-mediated stress response by MDM2 and MDM4, Cold Spring Harb. Perspect. Biol. 2 (1) (2010) a000968.
[10] Y. Haupt, R. Maya, A. Kazaz, M. Oren, Mdm2 promotes the rapid degradation of p53, Nature 387 (6630) (1997) 296–299.
[11] A. Shvarts, M. Bazuine, P. Dekker, Y.F. Ramos, W.T. Steegenga, G. Merckx, R.C. van Ham, W. van der Houven van Oordt, A.J. van der Eb, A.G. Jochemsen, Isolation and identification of the human homolog of a new p53-binding protein, Mdmx, Genomics 43 (1) (1997) 34–42.
[12] F. Toledo, G.M. Wahl, Regulating the p53 pathway: in vitro hypotheses, in vivo veritas, Nat. Rev. Cancer 6 (12) (2006) 909–923.
[13] H. Kawai, V. Lopez-Pajares, M.M. Kim, D. Wiederschain, Z.M. Yuan, RING domain-mediated interaction is a requirement for MDM2's E3 ligase activity, Cancer Res. 67 (13) (2007) 6026–6030.
[14] J.C. Marine, A.G. Jochemsen, Mdmx as an essential regulator of p53 activity, Biochem. Biophys. Res. Commun. 331 (3) (2005) 750–760.
[15] L.B. Gansmo, P. Romundstad, E. Birkeland, K. Hveem, L. Vatten, S. Knappskog, P.E. Lonning, MDM4 SNP34091 (rs4245739) and its effect on breast-, colon-, lung-, and prostate cancer risk, Cancer Med. 4 (12) (2015) 1901–1907.
[16] F. Gao, X. Xiong, W. Pan, X. Yang, C. Zhou, Q. Yuan, L. Zhou, M. Yang, A regulatory MDM4 genetic variant locating in the binding sequence of multiple MicroRNAs contributes to susceptibility of small cell lung Cancer, PLoS One 10 (8) (2015) e0135647.
[17] A.H. de Oliveira Reis, I.N. de Carvalho, P.B. de Sousa Damasceno, S.E. Ferman, E. Lucena, J.S. Lopez-Camelo, H.N. Seuanez, F.R. Vargas, Influence of MDM2 and MDM4 on development and survival in hereditary retinoblastoma, Pediatr. Blood Cancer 59 (1) (2012) 39–43.
[18] M. Hashemi, S. Sanaei, S.M. Hashemi, E. Eskandari, G. Bahari, Association of single nucleotide polymorphisms of the MDM4 gene with the susceptibility to breast Cancer in a southeast iranian population sample, Clin. Breast Cancer (2018).
[19] Z. Lu, H. Zhang, Y. Tao, X. Li, G. Li, MDM4 genetic variants predict HPV16-positive tumors of patients with squamous cell carcinoma of the oropharynx, Oncotarget 8 (49) (2017) 86710–86717.
[20] M.Y. Wang, M. Jia, J. He, F. Zhou, L.X. Qiu, M.H. Sun, Y.J. Yang, J.C. Wang, L. Jin, Y.N. Wang, Q.Y. Wei, MDM4 genetic variants and risk of gastric cancer in an Eastern Chinese population, Oncotarget 8 (12) (2017) 19547–19555.
[21] G. Pesole, F. Mignone, C. Gissi, G. Grillo, F. Licciulli, S. Liuni, Structural and functional features of eukaryotic mRNA untranslated regions, Gene 276 (1-2) (2001) 73–81.
[22] E.R. Jupe, A.A. Badgett, B.R. Neas, M.A. Craft, D.S. Mitchell, R. Resta, J.J. Mulvihill, C.E. Aston, L.F. Thompson, Single nucleotide polymorphism in prohibitin 3' untranslated region and breast-cancer susceptibility, Lancet 357 (9268) (2001) 1588–1589.
[23] P.M. Wong, Q. Yuan, H. Chen, B.M. Sultzer, S.W. Chung, A single point mutation at the 3'-untranslated region of Ran mRNA leads to profound changes in lipopolysaccharide endotoxin-mediated responses, J. Biol. Chem. 276 (35) (2001) 33129–33138.
[24] Z. Lu, E.M. Sturgis, L. Zhu, H. Zhang, Y. Tao, P. Wei, Q. Wei, G. Li, Mouse double minute 4 variants modify susceptibility to risk of recurrence in patients with squamous cell carcinoma of the oropharynx, Mol. Carcinog. 57 (3) (2018) 361–369.

[25] A.H. Reis, F.R. Vargas, B. Lemos, More epigenetic hits than meets the eye: microRNAs and genes associated with the tumorigenesis of retinoblastoma, Front. Genet. 3 (2012) 284.
[26] T. Saha, R.K. Kar, G. Sa, Structural and sequential context of p53: a review of experimental and theoretical evidence, Prog. Biophys. Mol. Biol. 117 (2-3) (2015) 250–263.
[27] A. Shvarts, W.T. Steegenga, N. Riteco, T. van Laar, P. Dekker, M. Bazuine, R.C. van Ham, W. van der Houven van Oordt, G. Hateboer, A.J. van der Eb, A.G. Jochemsen, MDMX: a novel p53-binding protein with some functional properties of MDM2, EMBO J. 15 (19) (1996) 5349–5357.
[28] K. Lehmann, P.M. Schneider, Differences in the molecular biology of adenocarcinoma of the esophagus, gastric cardia, and upper gastric third, Recent Results Cancer Res. 182 (2010) 65–72.
[29] G.L. Bond, W. Hu, E.E. Bond, H. Robins, S.G. Lutzker, N.C. Arva, J. Bargonetti, F. Bartel, H. Taubert, P. Wuerl, K. Onel, L. Yip, S.J. Hwang, L.C. Strong, G. Lozano, A.J. Levine, A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans, Cell 119 (5) (2004) 591–602.
[30] N.A. Laurie, S.L. Donovan, C.S. Shih, J. Zhang, N. Mills, C. Fuller, A. Teunisse, S. Lam, Y. Ramos, A. Mohan, D. Johnson, M. Wilson, C. Rodriguez-Galindo, M. Quarto, S. Francoz, S.M. Mendrysa, R.K. Guy, J.C. Marine, A.G. Jochemsen, M.A. Dyer, Inactivation of the p53 pathway in retinoblastoma, Nature 444 (7115) (2006) 61–66.
[31] N.A. Laurie, C.S. Shih, M.A. Dyer, Targeting MDM2 and MDMX in retinoblastoma, Curr. Cancer Drug Targets 7 (7) (2007) 689–695.
[32] Y. Jiao, Z. Jiang, Y. Wu, X. Chen, X. Xiao, H. Yu, A functional polymorphism (rs937283) in the MDM2 promoter region is associated with poor prognosis of retinoblastoma in chinese han population, Sci. Rep. 6 (2016) 31240.
[33] R. Chen, S. Liu, H. Ye, J. Li, Y. Du, L. Chen, X. Liu, Y. Ding, Q. Li, Y. Mao, S. Ai, P. Zhang, W. Ma, H. Yang, Association of p53 rs1042522, MDM2 rs2279744, and p21 rs1801270 polymorphisms with retinoblastoma risk and invasion in a Chinese population, Sci. Rep. 5 (2015) 13300.
[34] T. Sun, G.S. Lee, W.K. Oh, M. Pomerantz, M. Yang, W. Xie, M.L. Freedman, P.W. Kantoff, Single-nucleotide polymorphisms in p53 pathway and aggressiveness of prostate cancer in a Caucasian population, Clin. Cancer Res. 16 (21) (2010) 5244–5251.