The Functional TP53 rs1042522 and MDM4 rs4245739 Genetic Variants Contribute to Non-Hodgkin Lymphoma Risk

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Abstract

As a heterogeneous kind of malignances, Non-Hodgkin lymphoma (NHL) is the most common hematologic cancer worldwide with the significantly increased morbidity in China. Accumulated evidences demonstrated that oncoprotein MDM4 plays a crucial role in the TP53 tumor suppressor signaling pathway. An rs4245739 A>C polymorphism locating in the MDM4 3'-untranslated region creates a miR-191 target site and results in allele-specific MDM4 expression. In this study, we examined the association between this polymorphism as well as the TP53 Arg72Pro (rs1042522 G>C) genetic variant and Non-Hodgkin Lymphoma (NHL) risk in a Chinese Han population. Genotypes were determined in 200 NHL cases and 400 controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression. We found significantly increased NHL risk among carriers of the Pro/Pro genotype. We also observed a significantly decreased NHL risks among carriers of the MDM4 rs4245739 C allele compared with those with the A allele in Chinese (P = 0.014 for the AC genotype). Stratified analyses revealed the associations between these SNPs and NHL risk are especially noteworthy in young or male individuals. Additionally, the associations are much pronounced in NHL patients with B-cell lymphomas or grade 3 or 4 disease. Our results indicate that the TP53 Arg72Pro and the MDM4 rs4245739 polymorphisms contribute to NHL susceptibility and support the hypothesis that genetic variants in the TP53 pathway genes can act as important modifiers of NHL risk.

Introduction

As a heterogeneous group of malignances, Non-Hodgkin lymphoma (NHL) is the most common hematologic cancer worldwide with the significantly increased morbidity in China [1,2]. In 2012, the estimated incidence of NHL in China is 41171 cases. NHL derived from T cells or B cells is named as T-cell lymphomas (TCLs) or B-cell lymphomas (BCLs), respectively. TCLs and BCLs are abnormally differentiated from the precursor lymphocytes in different developmental stages. Immune deficiencies and some environmental factors have been identified to be involved in the pathogenesis of certain types of NHL, including human T-cell leukemia/lymphoma virus type 1, human immunodeficiency virus, Epstein-Barr virus and Helicobacter pylori [3–7]. Moreover, it has been shown that genetic makeup may also play important part during NHL development [8–12]. TP53 is one of most important tumor suppressors in human cells, which is essential in maintaining genomic stability and controlling cell growth as well as apoptosis [12,13]. As a key regulator of the TP53 tumor suppressor signaling pathways, MDM2 could lead to degradation of TP53 through the ubiquitin-proteasome pathway [14,15]. MDM4 is a structurally homologous protein of MDM2 and can cooperate with MDM2 to inhibit TP53 activities [16,17]. After interacting with MDM2 protein through the RING finger domain, MDM4 could repress degradation of MDM2 protein [17,18]. Transgenetic mice with overexpressed MDM4 showed spontaneous tumorigenesis, demonstrating that MDM4 is an important oncoprotein in vivo [19]. A functional single nucleotide polymorphism (SNP) (rs4245739 A>C) in the 3'-untranslated region (3'-UTR) of MDM4 has been identified, which creates a target binding site of miR-191 [20]. In
obtained from each subject. This study was approved by the Institutional Review Board of Shandong Cancer Hospital, Shandong Academy of Chinese. This study was approved by the Institutional Review Board of Shandong Cancer Hospital, Shandong Academy of Medical Sciences (Jinan, Shandong Province, China) and sex- and age-matched (±5 years) 400 controls. Patients were recruited between June 2009 and January 2014 at Shandong Cancer Hospital. Control subjects were randomly selected from a pool of 4500 individuals from a community cancer-screening program for early detection of cancer conducted in Jinan city during the same time period as the patients were collected. The diagnosis of all patients was histologically confirmed. All subjects were ethnic Han Chinese. This study was approved by the Institutional Review Board of Shandong Cancer Hospital, Shandong Academy of Medical Sciences. At recruitment, written informed consent was obtained from each subject.

SNP genotyping
PCR-based restriction fragment length polymorphism (RFLP) was used to determine TP53 Arg72Pro and MDM4 rs4245739 A>C genotypes as previously reported [22,23]. A 15% random sample was tested by different person, and the reproducibility was 99.8%. Moreover, a 5% random sample was also detected by Sanger sequencing, and the reproducibility was 100% (Figures S1 and S2).

Statistical analyses
The differences in demographic variables and genotype distributions of TP53 Arg72Pro and MDM4 rs4245739 SNPs between NHL patients and controls were examined via Pearson’s χ² test. Associations between TP53 Arg72Pro genotypes or MDM4 rs4245739 genotypes and NHL susceptibility were calculated by OR and their 95% CIs using the unconditional logistic regression model. All ORs were adjusted for age and sex, where it was appropriate. We tested the null hypotheses of multiplicative gene-gene or gene-covariate interaction and evaluated departures from multiplicative interaction models by including main effect variables and their product terms in the logistic regression model [28,29]. A P value of less than 0.05 was used as the criterion of statistical significance, and all statistical tests were two-sided. All analyses were performed with SPSS software package (Version 16.0, SPSS Inc., Chicago, IL).

Results
No statistically significant differences were found between NHL patients and controls for the case-control set in terms of median age and sex distribution (both P>0.05), which indicating that the frequency matching was adequate (Table 1). For NHL patients, 50 (25.0%) patients were classified into T-cell lymphoma and 150 (75.0%) were classified into B-cell lymphoma. Among cases with B-cell lymphoma, there were 133 (66.5%) patients with diffuse large B-cell lymphoma, 21 (10.5%) patients with follicular lymphoma, 20 (10.0%) patients with marginal zone lymphoma, 11 (2.3%) patients with chronic lymphocytic leukemia/small lymphocytic lymphoma and the remaining 15 (7.5%) other tumors, respectively (Table 1).

The allelic and genotype frequencies of TP53 Arg72Pro and MDM4 rs4245739 A>C SNPs were showed in Table 2. For the TP53 Arg72Pro polymorphism, the TP53 72Pro allele frequency was 0.383 among healthy controls and 0.483 among NHL patients. The frequency for the MDM4 rs4245739 C allele was 0.069 among healthy controls and 0.033 among NHL patients. All observed genotype frequencies in both controls and cases conform to Hardy-Weinberg equilibrium. We then compared distributions of these TP53 and MDM4 genotypes among NHL cases and controls. The frequencies of TP53 Arg/Arg, Arg/Pro and Pro/Pro genotypes among NHL patients were significantly different from those among controls (χ² = 11.29, P = 0.004, df = 2). The frequencies of MDM4 rs4245739 AA, AC and CC genotypes among NHL patients were also significantly different from those among controls (χ² = 6.76, P = 0.034, df = 2).

Associations between genotypes of TP53 Arg72Pro and MDM4 rs4245739 A>C SNPs and NHL risk were then calculated (Table 2). A significantly increased risk of developing NHL was associated with the TP53 Arg/Arg genotype (OR = 1.73, 95% CI = 1.16–2.57, P = 0.007) or the Pro/Pro genotype (OR = 2.18, 95% CI = 1.32–3.59, P = 0.002) compared with the TP53 Arg/Arg genotype. The MDM4 rs4245739 C allele was showed to be a protective allele. Individuals having the rs4245739 AC genotype had an OR of 0.45 (95% CI = 0.24–0.85, P = 0.014) for developing NHL compared with individual having the rs4245739 AA genotype (Table 2). All ORs were adjusted for age and sex. We also examined whether there are gene-gene interaction between MDM4 and TP53 polymorphisms, but the results were negative (Pinteraction = 0.681).

The risk of NHL associated with the TP53 Arg72Pro or MDM4 rs4245739 genotypes was further examined by stratifying for age, sex, pathology and Ann Arbor stage (Table 3 and 4). In stratified analyses with age, the TP53 Arg/Arg and Pro/Pro or the MDM4 AC and CC genotypes were significantly associated with NHL risk in subjects aged 50 years or younger (TP53: OR = 2.46, 95% CI = 1.45–4.16, P = 0.001; MDM4: OR = 0.42, 95% CI = 0.18–0.99, P = 0.048), but not in subjects aged older than 50 years (TP53: OR = 1.36, 95% CI = 0.80–2.32, P = 0.263; MDM4: OR = 0.48, 95% CI = 0.19–1.21, P = 0.121). No significant gene-age interaction was observed for both SNPs (Pinteraction = 0.122 or 0.854). Compared with the TP53 Arg/Arg genotype, a significantly increased risk of NHL was associated with TP53 Arg/Pro and Pro/Pro genotypes both among males (OR = 1.72, 95% CI = 1.08–2.73, P = 0.023), and among females (OR = 2.16, 95% CI = 1.13–
However, compared with the MDM4 AA genotype, a significantly decreased risk of NHL was associated with the MDM4 AC and CC genotypes only among males (OR = 0.21, 95% CI = 0.08–0.54, \( P = 0.001 \)), but not among females (OR = 1.35, 95% CI = 0.52–3.47, \( P = 0.536 \)). There was a significant gene-sex interaction for MDM4 genotypes (\( P_{\text{interaction}} = 0.007 \)), but not for TP53 genotypes (\( P_{\text{interaction}} = 0.530 \)).

In the pathology-stratified or grade-stratified analyses, significantly elevated NHL risk was found in the TP53 Arg/Pro and Pro/Pro genotypes carriers only in BCL cases (OR = 2.02, 95% CI = 1.31–3.10, \( P = 0.001 \)) and cases with grade 3 or 4 disease.

### Table 1. Distribution of selected characteristics among Non-Hodgkin Lymphoma cases and controls.

| Variable          | Cases (n = 200) | Controls (n = 400) | \( P \) |
|-------------------|-----------------|-------------------|--------|
| No. (%)           | No. (%)         |                   |        |
| Age (year) b      |                 |                   | 0.564  |
| \( \leq 50 \)     | 103(51.5)       | 196(49.0)         |        |
| \( > 50 \)        | 97(48.5)        | 204(51.0)         |        |
| Sex               |                 |                   | 0.809  |
| Male              | 128(64.0)       | 260(65.0)         |        |
| Female            | 72(36.0)        | 140(35.0)         |        |
| Pathology         |                 |                   |        |
| T-cell            | 50(25.0)        |                   |        |
| B-cell            | 150(75.0)       |                   |        |
| DLBCL             | 133(66.5)       |                   |        |
| FL                | 21(10.5)        |                   |        |
| MZL               | 20(10.0)        |                   |        |
| CLL/SLL           | 11(5.5)         |                   |        |
| Others            | 15(7.5)         |                   |        |
| Ann Arbor stage   |                 |                   |        |
| 1+2               | 84(42.0)        |                   |        |
| 3+4               | 116(58.0)       |                   |        |

Note: DLBCL: diffuse large B-cell lymphoma, FL: follicular lymphoma, MZL: marginal zone lymphoma, CLL: chronic lymphocytic leukemia, SLL: small lymphocytic lymphoma.

**Two-sided \( x^2 \) test.

\(^1\)Median age of cases is 50 years.

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### Table 2. Associations between the TP53 rs1042522 Arg72Pro and MDM4 rs4245739 A>C genetic polymorphisms and Non-Hodgkin Lymphoma risk.

| Genotype          | Cases (n = 200) | Controls (n = 400) | OR* (95% CI) | \( P \) |
|-------------------|-----------------|-------------------|-------------|--------|
| No. (%)           | No. (%)         |                   |             |        |
| TP53 rs1042522 Arg72Pro |                 |                   |             |        |
| Arg/Arg           | 52(26.0)        | 157(39.3)         | 1.00 (Reference) |   |
| Arg/Pro           | 103(51.5)       | 180(45.0)         | 1.73(1.16–2.57) | 0.007 |
| Pro/Pro           | 45(22.5)        | 63(15.7)          | 2.18(1.32–3.59) | 0.002 |
| Pro allele frequency | 0.483           | 0.383             |             |        |
| MDM4 rs4245739 A>C |                 |                   |             |        |
| AA                | 187(93.5)       | 346(86.5)         | 1.00 (Reference) |   |
| AC                | 13(6.5)         | 53(13.2)          | 0.45(0.24–0.85) | 0.014 |
| CC                | 0(0)            | 1(0.3)            | NC           | NC     |
| C allele frequency | 0.033           | 0.069             |             |        |

Note: NHL: Non-Hodgkin Lymphoma, OR: odds ratio, 95%CI: 95% confidence interval, NC: not calculated.

**Data were calculated by logistic regression, adjusted for sex and age.

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Discussion

In the current study, we investigated the association between TP53 and MDM4 functional SNPs and NHL risk in a case-control design. We found significantly increased NHL risk among carriers of the TP53 Arg72Pro allele compared with those with the 72Arg allele. Also, we observed a significantly decreased NHL risk among carriers of the MDM4 rs4245739 C allele compared with those with the A allele in Chinese. These results are in line with functional relevance of TP53 Arg72Pro as well as MDM4 rs4245739 polymorphism [20–27].

There are several studies which have investigated association between the TP53 Arg72Pro SNP and NHL susceptibility. However, the results are inconsistent among different ethnic populations [30–32]. In Korean, Kim et al examined the association between this TP53 Arg72Pro polymorphism and NHL risk through a Korean large-scale case-control study (945 cases and 1700 controls) [30]. They found that the TP53 72Pro/Pro genotype was associated with increased risk of NHL (P = 0.04), which is consistent to our observations in Han Chinese. However, no such association between this polymorphism and NHL risk was found in European Caucasians [31,32]. The controversial results might be due to differences of ethnic background. Additionally, Weng Y et al evaluate the role of TP53 Arg72Pro polymorphism in development of hematological cancer through a meta-analysis [33]. They found that significantly increased non-Hodgkin lymphomas risk was found in TP53

Table 3. Association between TP53 rs1042522 Arg72Pro variant and NHL risk stratified by selected variables.

| Variable | TP53 Arg72Pro | pinteractionc |
|----------|---------------|---------------|
|          | Arg/Arga | Arg/Pro+ Pro/Proa | ORb (95% CI) | P |
| Age (year) | | | | |
| ≤50 | 26/89 | 77/107 | 2.46(1.45–4.16) | 0.001 |
| >50 | 26/68 | 71/136 | 1.36(0.80–2.32) | 0.263 |
| Sex | | | | |
| Male | 35/101 | 93/159 | 1.72(1.08–2.73) | 0.023 |
| Female | 17/56 | 55/84 | 2.16(1.13–4.10) | 0.019 |
| Pathology | | | | |
| T-cell | 16/157 | 34/243 | 2.50(0.78–2.90) | 0.226 |
| B-cell | 36/157 | 114/243 | 2.02(1.31–3.10) | 0.001 |

Note: NHL: Non-Hodgkin Lymphoma, OR: odds ratio, 95%CI: 95% confidence interval, NC: not calculated.

Table 4. Association between MDM4 rs4245739 A>C variant and NHL risk stratified by selected variables.

| Variable | MDM4 rs4245739 A>C | pinteractionc |
|----------|-------------------|---------------|
|          | AAa | AC+CCa | ORb (95% CI) | P |
| Age (year) | | | | |
| ≤50 | 96/167 | 7/29 | 0.42(0.18–0.99) | 0.048 |
| >50 | 91/179 | 6/25 | 0.48(0.19–1.21) | 0.121 |
| Sex | | | | |
| Male | 123/218 | 5/42 | 0.21(0.08–0.54) | 0.001 |
| Female | 64/128 | 8/12 | 1.35(0.52–3.47) | 0.536 |
| Pathology | | | | |
| T-cell | 45/346 | 5/54 | 0.77(0.28–2.10) | 0.816 |
| B-cell | 142/346 | 8/54 | 0.34(0.16–0.74) | 0.006 |

Note: NHL: Non-Hodgkin Lymphoma, OR: odds ratio, 95%CI: 95% confidence interval, NC: not calculated.

aNumber of case patients with genotype/number of control subjects with genotype.
bData were calculated by logistic regression, adjusted for sex and age, where it was appropriate.
cP values for gene-environment interaction were calculated using the multiplicative interaction term in SPSS software.

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Arg72Pro polymorphism heterozygote model (Arg/Pro vs. Arg/Arg; OR = 1.10, 95% CI = 1.02–1.35) and dominant model (Arg/Pro+Pro/Pro vs. Arg/Arg; OR = 1.18, 95% CI = 1.03–1.34). These results are in line with our findings, indicating that TP53 Arg72Pro polymorphism may contribute to NHL susceptibility.

Wynendaele et al. found that the rs4245739 genetic variant in the MDM4 3’UTR creates a miR-191 target site, which was associated with survival of Caucasian ovarian cancer patients [20]. Previously, we also found that this SNP contributes to risk of esophageal squamous cell carcinoma and breast cancer in Chinese populations [22,23]. These data are consistent with results that genetic variants located in miRNA target sites may function as a new class of regulators modifying cancer risk.

We expected that there should be a gene-gene interaction between MDM4 rs4245739 and TP53 Arg72Pro genetic variants since the functional TP53 codon 72 Arg>Pro change could depress the activities of TP53 in inducing apoptosis and suppressing transformation [24,25] and MDM4 can negatively regulate TP53 tumor suppression function [16,17]. However, we did not observe this interaction, which might be largely due to the relatively small sample size of the current study. Therefore, the findings of our case-control study warrant to be validated in a population-based prospective study in the future.

Considering the gene-gender interaction of the MDM4 polymorphism, we did find marginal interaction in our previous study on esophageal cancer ($P_{interaction} = 0.080$) [22]. However, in the current study, we only observed significant association between MDM4 rs4245739 SNP and NHL in males. The most possible explanation might be the relative small sample size of the current study. One hypothesis to explain this is that miR-191 is an estrogen-responsive miRNA [34]. Therefore, there might be much higher expression of miR-191 in female patients compared to male individuals. The high level of miR-191 in cells may greatly repress MDM4 expression and compromise its allele-differential regulation of MDM4. However, experimental evidences are still needed to support this hypothesis.

In summary, our data demonstrated that functional TP53 Arg72Pro and MDM4 rs4245739 polymorphisms were significantly associated with NHL risk in a Chinese population. The associations between SNPs and NHL risk are especially noteworthy in young or male individuals. Additionally, the associations are much pronounced in NHL patients with BCL or grade 3 or 4 disease.

Supporting Information

Figure S1 Genotyping of the TP53 Arg72Pro (rs1042522 G>C) genetic variant. Up panel, PCR-RFLP results. Low panel, DNA sequencing results. (PPTX)

Figure S2 Genotyping of the MDM4 rs4245739 A>C genetic variant. Up panel, PCR-RFLP results. Low panel, DNA sequencing results. (PPTX)

Author Contributions

Conceived and designed the experiments: MY CZ. Performed the experiments: CF JW. Analyzed the data: CF JW. Contributed reagents/materials/analysis tools: CY CZ XW CJ. Contributed to the writing of the manuscript: MY CZ.
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