Int J Clin Exp Pathol 2017;10(3):3735-3738
www.ijcep.com /ISSN:1936-2625/IJCEP0025564

Original Article

The polymorphism at the miRNA binding site of *GOLGA*$_7$ is associated with the Non-Hodgkin's lymphoma cancer risk

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Received February 4, 2016; Accepted July 12, 2016; Epub March 1, 2017; Published March 15, 2017

Abstract: Aims: MicroRNAs (miRNAs) binds to the 3'-untranslated regions (UTRs) of messenger RNAs and regulates cell differentiation, apoptosis and tumor genesis. Genetic polymorphisms at the 3'-UTRs alter the strength of miRNA binding and affect the behavior of miRNA. In this study, we analyzed a single-nucleotide polymorphism (SNP) of rs11337 within the miRNA seed region for the 3'-UTR of GOLGA$_7$ gene to assess its association with cancer risk of Non-Hodgkin's lymphoma (NHL) in a case-control study in Chinese population. Methods: rs11337 were genotyped with ligation detection reaction (LDR) method. The odds ratio (OR) and 95% confidence interval (95% CI) of rs11337 in NHL and healthy control were calculated using an unconditional logistic regression model. Result: rs11337 is associated with the cancer risk of NHL (OR=1.339, 95% CI 0.989-1.815), and cancer risk association was also confirmed in NHL subtype of diffuse large B-cell lymphoma (DLBCL) (OR=1.555, 95% CI 0.581-1.825). Conclusion: The SNP at the miRNA-binding site of GOLGA$_7$ 3'-untranslated regions influences cancer risk of NHL. The analysis of genetic polymorphisms in miRNA-binding sites may help to identify high-risk NHL patient subgroups.

Keywords: Non-Hodgkin's lymphomas, SNP, rs11337, cancer risk, GOLGA$_7$

Introduction

Non-Hodgkin's lymphoma (NHL) is the most common cause of cancer-related death and NHL incidence is almost doubled in the past few decades [1-3]. Diffuse large B-cell lymphoma (DLBCL), T-cell lymphoma (TCL), and follicular lymphoma (FL) are the most common NHL subtypes and composed of a heterogeneous group of tumors arising from B or T/NK cells at various stages of differentiation [4]. The NHL risk factors are complicated and may be involved in age, sex, genetic and etc [5, 6]. Recently, a couple of studies have linked polymorphisms in mature and precursor microRNA (miRNA) sequences, miRNA promoters and miRNA binding sites to differential risk for many human cancer [7].

miRNA is a type of non-coding small RNA and considered as an inherited factor. miRNAs are processed in the nucleus, exported to the cytoplasm and ultimately bound to the 3'untranslated region (3'-UTR) of their target mRNA by the RNA-induced-silencing-complex (RISC). Complementarities between miRNA and its target mRNA sequence reduce protein levels through RNA silencing. The miRNA-related single nucleotide polymorphisms (miR-SNPs) in the 3'-UTR region targeted by miRNAs may alter the expression of target genes and thereby affect the cancer risk. Recent reports linked polymorphisms in miRNA sequences, miRNA promoters, and miRNA binding sites to differential risk for many human cancers [7]. Those cSNPs may be used as biomarkers to predict the cancer risk and outcome for NHL patients [8-10].

In this study, we genotyped *RYR3* (rs1044129), *C14 or f101* (rs4901706), *KIAA0423* (rs1053667), *KRT81* (rs3660) among NHL patients and identified the potential cancer risk association between these SNPs and NHL development.

Table 1. The clinical characteristics of 388 NHL patients and 347 controls involved

	Value	NHL Patients n=388	Healthy Controls n=347	*P*-value
Age (year)				
	≤60	274	255	0.387
	>60	114	92	
Sex				
	Male	237	229	0.168
	Female	151	118	
Ann Arbor Stage				
	I/II	139		
	III/IV	249		
LDH				
	Abnormal	151		
	Normal	237		
Bone				
	Normal	296		
	Abnormal	92		
B Symptom				
	Yes	222		
	No	166		
Lymph node size (cm)				
	>5	89		
	≤5	299		

Materials and methods

Study population and ethic statement

388 NHL patients Blood samples were collected in the 2nd Hospital of Hebei Medical University from 2000 to 2007 and clinical profiles were obtained from all the subjects. 347 blood samples from healthy people were also collected. The NHL diagnosis was made according to the WHO Classification of Tumors [11]. All procedures were supervised and approved by the Human Tissue Research Committee of the 2nd Hospital of Hebei Medical University. Written consents were obtained from all the patients enrolled in this study for the collection of samples and subsequent analysis in this publication.

DNA extraction and genotyping of miRNA-SNPs

Genomic DNA was extracted from blood samples by using the Wizard Genomic DNA extraction kit (Promega, Madison, WI). miRNA-SNPs were genotyped by using the ligation detection reaction (LDR) method with the forward and reverse primers, 5'-CGCTGTATTTGGGAGAGAGTT-3' and 5'-CAGGCTGTAAAGTAACAAATGAG-3', to amplify DNA fragments flanking the site in the 3'-UTR of corresponding gene. Primers were designed specifically to the target sequence. PCR was performed using a PCR Master Mix kit according to the manufacturer's instruction (Promega, Madison, WI). The ligation was performed using the probes listed in **Table 1**, and the ligated products were separated using the ABI PRISM Genetic Analyzer 3730XL (Applied Biosystems; Life Technologies, Carlsbad, CA, USA). Polymorphisms were confirmed based on a 3-bp length for different alleles.

Statistical analysis

Hardy-Weinberg equilibrium analysis was employed to compare the observed and expected genotype frequencies. The χ^2 test was used to analyze dichotomous values, such as the presence or absence of an individual SNP in patients and healthy controls. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated using an unconditional logistic regression model. All of the statistical analyses were performed with the SPSS 19.0 software package (IBM SPSS Inc., Chicago, IL). Results with a P value of < 0.05 were considered statistically significant.

Results

Clinical characteristics

388 NHL patients ranging from stage I to IV and 347 controls were included in this study. The case group included 184 DLBCL, 146 Peripheral T-Cell Lymphoma (PTCL) and 58 FL patients. The size of tumor was measured by computed tomography (CT) scan and the serum lactate dehydrogenase (LDH) was seen as abnormal when it was above 500 U/L. Some clinical characteristics, such as the Ann Arbor Stage, the LDH level, bone marrow invasion and the presence of B symptoms, were shown in **Table 1**.

Association of rs11337 genotype with the NHL risk

The gene distribution of rs11337, a polymorphism at the miRNA binding site in 3'untrans-

Table 2. Correlation between rs11337 SNP and NHL cancer risk

Genetype/ allele	Case n (%)	Controls N (%)	*P*-value	OR	95% CI
G/G	264 (68.1)	213 (61.4)		1.000	
G/T	103 (26.5)	116 (33.4)	0.041	1.396	1.013-1.924
T/T	21 (5.4)	18 (5.2)	0.856	1.062	0.552-2.045
G/T+T/T	124 (31.9)	134 (38.6)	0.059	1.339	0.989-1.815

Table 3. Correlation between rs11337 SNP and NHL subtype

Genetype/allele	G/G	G/T+T/T	*P*-value	OR	95% CI
Controls N (%)	213	134			
PTCL N (%)	97	49	0.289	1.245	0.830-1.869
DLBCL N (%)	131	53	0.024	1.555	1.058-2.286
FL N (%)	36	22	0.921	1.029	0.581-1.825

lated region of *GOLGA*$_7$, was examined in 388 NHL patients and 347 controls. G/G, G/T and T/T genotype of the rs11337, frequencies in controls was 61.4%, 33.4% and 5.2%, and the distribution was fit in Hardy-Weinberg equilibrium analysis respectively. The case control study indicates the frequent allele of rs11337 GG is associated with a higher risk for NHL (**Table 2**).

Association of the rs11337 with the risk of NHL subtypes

The predictive power of HNL risk for rs11337 was also assessed among different NHL subtypes. For DLBCL, the patients with GG the genotype displayed a higher NHL risk at a statistically significant level with *P*=0.024. However, the predictive power of this miRNA-SNP for cancer risk was not statistically significant in PTCL and FL (**Table 3**).

Discussion

In this study, we analyzed the SNP of rs11337 within the miRNA seed region for 3'-UTR in GOLGA$_7$ gene to assess its association with cancer risk of NHL in Chinese population. The patients with the GG genotype of rs11337 in *GOLGA*$_7$ show an increased NHL risk than those with the GT or TT genotype in this case-control study. The *GOLGA*$_7$ rs11337 GG genotype is a predicted factor in NHL (*P*=0.059, OR=1.339, 95% CI 0.989-1.815). We also validated that *GOLGA*$_7$ rs11337 GG genotype is a predicted factor (*P*=0.024, OR=1.555, 95% CI 0.581-1.825) in DLBCL. Our report is the first study for the role of GOLGA$_7$ rs11337 in Chinese NHL patients.

GOLGA$_7$ gene encodes GCP16, a member of the golgin family and is involved in vesicular transporting from the Golgi to cell surface. GCP16 was reported to be a major component of DHHC9-GCP16 complex. It plays an important role in affecting cell apoptosis and regulating RAS signaling by post-translational acylating of RAS proteins [12, 13]. We showed that the SNP in miRNA seed region for the 3'-UTR of *GOLGA*$_7$ associates with clinical outcome of NHL patients, which suggested that variant miRNA binding ability might interference the *GOLGA*$_7$ transcript and GCP16 protein synthesis and lead to the development of NHL.

The miRNA pathway emerges as a crucial system for the regulation of tumorgenesis and miR-SNPs, related to the miRNA pathway. miRNA functions are affected by directly impacting miRNA expression levels or influencing the miRNA-target interaction [14]. For example, in our previous study, we showed that KRT81 rs3660 is associated with the survival of NHL patients. The polymorphism in rs3660 of KRT81 may affect the function of KRT81 through affecting the altered hybridization process in formatting of the RNA-miRNA binding structure [10].

The studies of SNPs in *GOLGA*$_7$ miRNA binding sites association with NHL development are still preliminary. The mechanism of rs11337 GG genotype affecting the expression of *GOLGA*$_7$ remains unknown although the patients with the GG genotype of rs11337 in *GOLGA*$_7$ showed a significantly increased NHL risk than those with the GT or TT genotype in this case-control study. It is necessary to compare the mRNA and protein levels of *GOLGA*$_7$ between GG genotype and GT/TT genotype group in future. In conclusion, the SNP rs11337 in *GOLGA*$_7$ was identified as an independent prognostic marker in Chinese NHL population. The analysis of genetic polymorphisms in miRNA binding sites of *GOLGA*$_7$ may help stratify high-risk population subgroups.

Disclosure of conflict of interest

None.

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References

[1] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[2] Jemal A. Global burden of cancer: opportunities for prevention. Lancet 2012; 380: 1797-1799.

[3] Cultrera JL, Dalia SM. Diffuse large B-cell lymphoma: current strategies and future directions. Cancer Control 2012; 19: 204-213.

[4] Perry AM, Mitrovic Z, Chan WC. Biological prognostic markers in diffuse large B-cell lymphoma. Cancer Control 2012; 19: 214-226.

[5] Manns A, Cleghorn FR, Falk RT, Hanchard B, Jaffe ES, Bartholomew C, Hartge P, Benichou J, Blattner WA. Role of HTLV-I in development of non-Hodgkin lymphoma in Jamaica and Trinidad and Tobago. The HTLV Lymphoma Study Group. Lancet 1993; 342: 1447-1450.

[6] Rastin M, Khoee AR, Tabasi N, Sheikh A, Ziaolhagh S, Esmaeeli E, Zamani S, Khazaee M, Mahmoudi M. Evaluation of HTLV-I and HCV Prevalence in Non-Hodgkin's Lymphoma. Iran J Basic Med Sci 2013; 16: 242-246.

[7] Fu A, Hoffman AE, Liu R, Jacobs DI, Zheng T, Zhu Y. Targetome profiling and functional genetics implicate miR-618 in lymphomagenesis. Epigenetics 2014; 9: 730-737.

[8] Wang C, Guo Z, Wu C, Li Y, Kang S. A polymorphism at the miR-502 binding site in the 3' untranslated region of the SET8 gene is associated with the risk of epithelial ovarian cancer. Cancer Genet 2012; 205: 373-376.

[9] Wong KY, So CC, Loong F, Chung LP, Lam WW, Liang R, Li GK, Jin DY, Chim CS. Epigenetic inactivation of the miR-124-1 in haematological malignancies. PLoS One 2011; 6: e19027.

[10] de Larrea CF, Navarro A, Tejero R, Tovar N, Díaz T, Cibeira MT, Rosiñol L, Ferrer G, Rovira M, Rozman M, Monzó M, Bladé J. Impact of MiRSNPs on survival and progression in patients with multiple myeloma undergoing autologous stem cell transplantation. Clin Cancer Res 2012; 18: 3697-3704.

[11] Sabattini E, Bacci F, Sagramoso C, Pileri SA. WHO classification of tumours of haematopoietic and lymphoid tissues in 2008: an overview. Pathologica 2010; 102: 83-87.

[12] Ohta E, Misumi Y, Sohda M, Fujiwara T, Yano A, Ikehara Y. Identification and characterization of GCP16, a novel acylated Golgi protein that interacts with GCP170. J Biol Chem 2003; 278: 51957-51967.

[13] Swarthout JT, Lobo S, Farh L, Croke MR, Greentree WK, Deschenes RJ, Linder ME. DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras. J Biol Chem 2005; 280: 31141-31148.

[14] Schuetz JM, Daley D, Graham J, Berry BR, Gallagher RP, Connors JM, Gascoyne RD, Spinelli JJ, Brooks-Wilson AR. Genetic variation in cell death genes and risk of non-Hodgkin lymphoma. PLoS One 2012; 7: e31560.