RESEARCH ARTICLE

Lack of Association between the *MiR146a* Polymorphism and Susceptibility to Thai Childhood Acute Lymphoblastic Leukemia

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Abstract

Background: MiRNAs, small non coding RNAs, play a role in the regulation of hematopoiesis, with effects on cell growth, differentiation, and apoptosis. In addition, MiRNAs are thought to play an important role in tumorigenesis. The miR146a G>C polymorphism can lead to alteration of miR146 expression, which appears to be associated with development and progression of several cancers. This study aimed to investigate the association of the miRNA146a (rs2910164) G>C polymorphism and susceptibility to childhood acute lymphoblastic leukemia (ALL) and clinical outcomes. Materials and Methods: Totals of 100 childhood ALL patients and 200 healthy children were studied for miR146a polymorphisms using polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP). Results: The frequency of the miR146a G allele in controls was 0.40 compared with 0.38 in ALL patients. There was no association between miRNA146a (rs2910164) G>C polymorphism and susceptibility to childhood ALL (OR=1.484, 95%CI=0.712-3.093, p=0.290). Moreover, the frequencies of miR146a (rs2910164) G>C polymorphism were not associated with demographic data and clinical outcomes in ALL cases. Conclusions: The miRNA146a polymorphism was not significantly associated with susceptibility to Thai childhood ALL or any clinico-pathological variables.

Keywords: Acute lymphoblastic leukemia - miRNA146a - polymorphism - susceptibility

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children. The peak incidence of ALL occurs between age 2 and 5 years (Inaba, 2013). The improvement of ALL therapy is one of the great successes with long-term survival achieved in over 80% of patients (Pui et al., 2006, Vrooman and Silverman, 2009). However, the remaining cases cannot be cured and has poor prognosis. ALL is a heterogenous leukemia based on the type and stage of lymphoblast. The molecular biology of ALL is not completely understood. Genome-wide association studies show that genetic polymorphisms of some susceptibility genes are associated with the development of childhood ALL (Treviño LR et al., 2009).

Recently, it is discovered that microRNAs (MiRNAs) play a role in the regulation of hematopoiesis (Caterine Labbaye and Ugo, 2012). MiRNAs, small non coding RNAs about 17-22 nucleotide in length, regulate gene expression by translational repression or mRNA degradation (Bartel, 2004). MiR 146a has an important role for negative regulation of acute responses during the activation of the innate immune system and plays a role in the regulation of most biology processes such as differentiation and surveillance of hematopoietic cells. (Caterine and Ugo 2012; Wang et al., 2012). The miR 146a polymorphism (rs2910164) involves a G > C nucleotide substitution which causes change from a G:U pair to a C:U mismatch in the stem structure of *miRNA 146a* precursor that results in a reduced amount of mature *miR146a*. (Cong et al., 2011; Palmieri et al., 2014). Up or down regulation of miRNA-146a is observed in human disorders, such as inflammatory diseases and cancers. It has been shown that miRNA-146a (rs2910164) can directly inhibit the expression of IRAK1 and IRAF6, impair nuclear factor (NF)-kB activity, and suppress the expression of NF-kB target genes, such as IL-6, IL-8, IL-Iβ, and TNF-α (Caterine and Ugo, 2012; Wang et al., 2012).

The *miR146a* G>C polymorphism was associated with increased risk of cancer including breast cancer (Hai Lian et al., 2012; Qi P, Wang L et al,2015), hepatocellular carcinoma (Zhaoming Wang et al., 2014). However, there

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was a report of meta-analysis suggested an increased risk between *miR146a* rs2910164 GG genotype and gastric cancer susceptibility. (Zhong et al., 2014) and another findings suggested that *miRNA146a* polymorphism was not associated with several cancers including oral cancer (Yin Hung Chu et al., 2012), colorectal cancer (Min KT et al., 2012; Wei Du et al., 2014) and lung cancer (Tian T et al., 2009). The conflict results may be due to ethnic sampling and number of cases.

Hasani SS et al., evaluated the possible relationship between two miRNA polymorphisms, included hsa-*miRNA146a* and hsa-*miRNA499* with the risk of childhood ALL in Iranian population. They found that the G>C variant of hsa-*miRNA146a* significantly increased the risk of ALL. With respect to hsa-*miRNA499*, no significant difference in allele and genotype frequencies between ALL patients and controls was reported (Hassani SS et al., 2014). In Thailand, there is no report of *miRNA146a* polymorphism and acute lymphoblastic leukemia.

Therefore, the genotyping of *miRNA 146a* (rs2910164) was determined in this study and the gene frequency was compared between childhood ALL and healthy children. Moreover, the association of single nucleotide polymorphism of *miRNA 146a* (rs2910164) with clinical data in childhood ALL were studied.

Materials and Methods

Specimens

In this retrospective study, Complete blood count was performed in 100 ALL patients and 200 normal healthy children at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Thailand. Patients were classified by risk-based assignment protocol (Smith et al., 1996), assessed by initial white blood cell counts, French-American-British morphology and lymphomatous disease. Moreover clinical and demographic data including age at diagnosis, sex, immunophenotype and chromosome abnormalities were retrospectively studied. This study was approved by the Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University and inform consent was obtained from all the participants.

DNA isolation

Genomic DNA in 200 healthy controls and 100 ALL patients was isolated from 3 ml EDTA blood by proteinase K digestion and salting-out method (Miller SA et al.,1988). Dissolved pellet in sterile water was quantitated by a measurement of nano drop spectrophotometer and kept at -20°C prior to use.

Genotyping of the miR146a (G>C) polymorphism

The *miR146a* (G>C) polymorphism was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis. Genotyping was done using forward primer 5'-CATGGGTTGTGTCAGTGTCAGAGCT-3' and reverse primer 5'- TGCCTTCTGTCTCCAGTCTT CCAA-3' as described previously (Cong et al., 2011). DNA was amplified in a 25 μl reaction mixture containing 100 ng of genomic DNA, 20 μM of each primer, 5 μl of 10X buffer, 2.5 μl of 5X Q-solution, 10mM of each dNTP and 1 unit of hotstar taq DNA polymerase (Qiagen, Germany). Polymerase chain reaction was performed in a PCR thermal cycle (Biomatra, Germany) using the following conditions. Initial denaturation 94°C for 5 min, followed by denaturation at 94°C for 30 s, annealing at 64°C for 40s, and extension at 72°C for 45s. After completion of 35 cycles, a final extension step was carried out at 72o C for 5 min. PCR amplicon sized were identified by 2% agarose gel electrophoresis. PCR amplicons were digested by SacI at 37°C for 16 hrs. The product sizes after restriction digested were verified on 10% polyacrylamide gel electrophoresis, stained with ethidium bromide and visualized using the gel documentation. The product presented 3 different patterns: a single 147 bp fragment for the GG genotype; two fragments of 122 and 25 bp for the CC genotype ; and three fragments of 147, 122 and 25 bp for the GC genotype.

Statistical analysis

The associations between the demographic data, the distribution of the *miR 146a* genotypes and risk group of the patients were calculated by binary logistic regression analysis using SPSS version 17.0 program (SPSS Inc., Chicago, USA). Comparisons between ALL cases and normal control group were analyzed by Chi-square test. Crude odds ratios (OR) and 95% confidence intervals (CI) were also calculated. P values less that 0.05 were considered statistically significant.

Results

This study was consisted of 100 ALL patients (38 females and 62 males) and 200 healthy children (98 females and 102 male). The demographic data of ALL patients and normal controls as well as clinical data of ALL patients were shown in Table 1. The allele frequencies for *miR146a* genotypes were determined in childhood ALL

Table 1. Demographic and Clinical Data of ALL Patients and Controls

Demographic characteristics	n= 100 (cases)	n=200 (controls)
Gender		
Males	62 (62%)	102 (51%)
Females	38 (38%)	98 (49%)
Age distribution		
1-9 years	69 (71.88%)	176 (88%)
≥10 years	27 (28.12%)	24 (12%)
Initial WBC count		
<50,000 cell /μl	73 (80.22%)	200 (100%)
>50,000 cell /μl	18 (19.78%)	0
Risk classification		
Standard risk or low risk	86 (87.76%)	-
High Risk	12 (12.24%)	-
Immunophenotype		
T cell	10 (10.20%)	-
Non T cell	88 (89.80%)	-
Early pre-B	77 (87.5%)	-
pre-B	10 (11.36%)	-
Mixed	1 (1.14%)	-

Table 2. Distribution MiR146a G>C Genotype in ALL, Compared with Controls

Genotypes	ALL (%)	Controls (%)	OR (95%CI)	P-value
MiR146a G>C				
G/G	11 (11)	31 (15.5)	1.00 (Reference)	
G/C	54 (54)	96 (48)		0.24
C/C	35 (35)	73 (36.5)		0.46
G/C+C/C	89 (89)	169 (84.5)	1.484 (0.712-3.093)	0.29
Allele frequency				
MiR146a G	0.38	0.395		
MiR146a C	0.62	0.605		

Table 3. Demographic Data of ALL Patients of MiR146a G>C genotypes

Demographic data	Total patients	MiR146a genotype		P-value
		G/G	G/C or C/C	
Sex				
Male	62	5 (8.06)	57 (91.94)	0.191
Female	38	6 (15.79)	32 (84.21)	
Age at diagnosis				
1-9 years	69	10 (14.49)	59 (85.51)	0.125
≥10 years	27	1 (3.70)	26 (96.3)	
Initial WBC count				
<50,000 cell /μl	73	6 (8.22)	67 (91.78)	0.249
>50,000 cell /μl	18	3 (16.67)	15 (83.33)	
Risk classification				
Standard risk or low risk	86	10 (11.63)	76 (88.37)	0.598
High Risk	12	1 (8.33)	11 (91.67)	
Immunophenotype				
Non T cell	88	11 (12.50)	77 (87.50)	0.235
T cell	10	0 (0)	10 (100)	

and controls. The allele frequencies of *miR146a* C allele in controls was 0.605 compared with 0.62 in ALL patients. There was no association between *miRNA146a* (rs2910164) G>C polymorphism and susceptibility to childhood ALL (OR=1.484, 95%CI=0.712-3.093, p=0.290) (Table2). Moreover, the frequencies of *miRNA146a* (rs2910164) G>C polymorphism in ALL cases were not associated with demographic data and clinical outcomes including gender, age at diagnosis, initial WBC count, risk classification and immunophenotype. (Table 3).

Discussion

Recently, it has been shown that small non coding miRNAs are important components of complex gene regulatory. MicroRNAs play roles in development and cellular processes such as cell proliferation, differentiation, apoptosis and tumorigenesis (Hwang and Mendell, 2006; Wang et al., 2012). MicroRNAs can be classified as oncogenes and tumor suppressor genes. It has been reported that the expression levels of oncogenic microRNAs are increased in cancer patients, whereas tumor suppressor microRNAs are decreased (Garcon et al, 2010). Jazdaewski et al., 2008, firstly reported that the *miRNA146a* (rs2910164) G>C was associated with increased susceptibility to thyroid papillary carcinoma. They found that C allele of mature *miRNA146a* would cause decrease inhibition target genes including TRAF6, IRAK1 (Jazdaewski et al., 2008). Moreover, *miRNA146a* acts as a mediator of the pro-apoptotic transcription factor nuclear-kappaB (NF-kB) (Tagnov et al., 2006).

MiRNA146a (rs2910164) G>C polymorphism was associated with increased risk of cancer including breast cancer (Qi P, Wang L et al,2015), hepatocellular carcinoma (Zhaoming Wang et al., 2014). On the contrary, it was reported that the rs2910164 CG or GG genotype was associated with a significantly decreased risk for lung cancer compared to that of the CC genotype (Hyo, 2014). Moreover, it was found that *miR146a* rs2910164 GG genotype was significantly gastric cancer susceptibility. (Zhong et al., 2014). The conflict of these findings may depend on sample size, ethnic groups and the type of cancer.

Recently, one report from Iran study the association between SNP (rs2910164), *miR146a* G>C and susceptibility to acute lymphoblastic leukemia (ALL). They found that the rs2910164 G>C variant of *miR146a* significantly increased the risk of ALL. (Hasani SS.et al., 2014).

In this study, *miRNA146a* G>C polymorphism was not significantly associated with susceptibility to childhood ALL. The different results from the previous study of *miR146a* G>C polymorphisms in ALL, may be due to different ethnic groups. In control subjects, the allele frequency of *miR146a* G allele was 0.395 and *miR146a* C allele was 0.605. The data of these gene frequencies were similar to SNP database in Asian population but different from European groups. It was noted that the frequency of C allele is high in control group. Moreover, allele frequency in *miR146a* G allele and *miR146a* C allele in ALL was very similar to control group.

There were no statistically significant differences between *miRNA146a* (rs2910164) G>C polymorphism in terms of gender, age at diagnosis, initial WBC count, risk classification and immunophenotype. This may be due to the small sample size. However, it is interesting that polymorphism of *miR146a* (GC/CC) was found in 26 of 27 cases (96.3%) of ≥ 10 years old ALL patients and in all of 10 cases (100%) in T cell ALL patients. There was some evidence about the role of miR-146a and T cell response (Lili et al., 2012). This is the pathogenesis that why *miR146a* (GC/CC) polymorphism is found in all cases

of T cell ALL. However, it was not significant because the low incidence of T cell ALL contributed to small sample size of T cell ALL. This study is only the preliminary report of clinical outcome. The larger sample size should be done in the future for the clinical outcome study.

In conclusion, *miRNA146a* polymorphism was not significantly associated with susceptibility in Thai childhood ALL and was not significantly associated with clinico-pathological variables.

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