

# Polymorphisms in MicroRNA-Related Genes Are Associated With Survival of Patients With T-Cell Lymphoma

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Disclosures of potential conflicts of interest may be found at the end of this article.

**Key Words.** MicroRNAs • Polymorphisms • T-cell lymphoma • Survival

## ABSTRACT

**Objective.** Elaborate evaluation of prognosis of T-cell lymphoma (TCL) is vital for current therapy and future stratified and individualized therapy. MicroRNAs (miRNAs) play important roles in cancer development and prognosis. We aimed to assess the effects of single nucleotide polymorphisms (SNPs) in miRNA-related genes on the survival of patients with TCL.

**Patients and Methods.** We genotyped 13 SNPs selected from 12 miRNA-related genes in 220 TCL patients and explored the association of SNPs with survival.

**Results.** Among the 13 SNPs, four (*DROSHA* rs6877842, *DICER* rs3742330, *mir149* rs2292832, and *mir499* rs3746444) were significantly associated with TCL survival after adjusting for subtype and International Prognostic Index score. In stratified

analyses, all four SNPs remained significantly associated with survival in patients with mature T type. Of the four SNPs, only *mir149* rs2292832 was not significantly associated with survival in patients with an International Prognostic Index score of 0–1. Furthermore, a dose-dependent cumulative effect of the four SNPs on TCL survival was observed by counting the number of unfavorable genotypes. Survival tree analysis also showed higher order interactions between these SNPs.

**Conclusion.** The results suggested that miRNA-related polymorphisms are associated with survival of TCL patients; thus, they may be used individually and jointly to predict survival of patients with TCL. *The Oncologist* 2014;19:243–249

**Implications for Practice:** Besides the characteristics of a tumor itself, genetic polymorphisms in relevant genes are considered to be important in influencing the clinical outcomes of patients. Previous studies have suggested that microRNAs are associated with survival of patients with T-cell lymphoma (TCL); therefore, we performed association analyses of 13 carefully selected polymorphisms in microRNA-related genes. The results suggested that four polymorphisms in microRNA-related genes are associated with survival of patients with TCL. These polymorphisms may be used to predict the survival of patients with TCL individually or collectively. Although the results are exploratory, they provide clues to warrant further investigation.

## INTRODUCTION

T-cell non-Hodgkin lymphomas (TCLs) are uncommon malignancies accounting for approximately 12% of all lymphomas [1]. They are composed of a heterogeneous group of diseases and usually have poor prognosis [2, 3]. Currently, the International Prognostic Index (IPI) is widely used to predict prognosis of TCL. The survival probability decreases as the IPI score increases, and patients are divided into low-risk (IPI score 0–1), intermediate-risk (IPI score 2–3), and high-risk (IPI score 4–5) groups according to their survival prospects [2]. In addition, histological subtype is strongly associated with survival of TCL patients [3]. However, these parameters are far from satisfactory for guiding stratified or individualized therapy. It is obvious that complementary prognostic indices for TCL using pretherapeutic characteristics are urgently needed.

MicroRNAs (miRNAs) are a class of endogenous, small, noncoding RNA molecules that participate in diverse biological

processes by regulating gene expression. miRNAs are generated by a two-step pathway. They are first transcribed by RNA polymerase II into primary miRNAs (pri-miRNAs). These pri-miRNA transcripts are processed in the nucleus by DROSHA RNase to produce the precursor miRNAs (pre-miRNAs) [4, 5]. The pre-miRNAs are then translocated to the cytoplasm where the pre-miRNAs are cleaved by DICER, leading to the production of mature miRNAs [6]. The mature miRNAs cooperate with the RNA-induced silencing complex comprising GEMIN3, GEMIN4, TRBP, AGO1, and AGO2, resulting in messenger RNA cleavage or translational repression [7]. Several recent studies have demonstrated that aberrations of key genes in the miRNA biogenesis pathway can modify the response of TCL cells to chemotherapy and then affect the survival of patients with TCL [8–10].

Single nucleotide polymorphisms (SNPs) have been shown to be associated with the survival of patients with TCL [11, 12].

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However, data are sparse for miRNA biogenesis pathway genes and miRNA genes. Recent studies have suggested that SNPs in these genes can affect gene functions and miRNA expression [13] and, thus, are associated with survival of cancer patients. To provide potential new biomarkers for predicting prognosis of TCL, we therefore evaluated the effects of 13 SNPs in miRNA biogenesis pathway genes and miRNA genes on the survival of patients with TCL individually and jointly.

## MATERIALS AND METHODS

### Study Patients

TCL patients ( $n = 220$ ) were recruited from Chongqing Southwest Hospital and Beijing Cancer Hospital between January 1992 and September 2009. One hundred fifty-eight of these patients were the subject of a previous report [14]. The diagnosis of TCL was based on pathological examination and clinical manifestations. All patients were non-blood-related Han Chinese and were mainly treated with a CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)-based regimen as the first-line chemotherapy. Here, we classified TCL as natural killer/T-cell lymphoma, peripheral T-cell lymphoma not otherwise specified, T-lymphoblastic lymphoma/leukemia, angioimmunoblastic T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, mycosis fungoides, enteropathy-type T-cell lymphoma, and Lennert lymphoma. The stage of tumor was assessed according to the Ann Arbor system [15]. At recruitment, demographic information and clinical characteristics were collected from each patient via clinical record. Data on whether and when a patient had died were obtained from inpatient and outpatient records, patient or family contact, or local Public Security Census Register Office. All patients provided written informed consent, and the study was approved by the Institutional Review Boards of both the Southwest Hospital of Third Military Medical University and Chinese Academy of Medical Sciences Cancer Institute.

### Gene/SNP Selection

We selected 13 SNPs in miRNA biogenesis pathway genes and miRNA genes according to the following criteria: (a) they have a reported minor allele frequency  $>0.01$  in the Chinese population; (b) they reside in functional regions, including exons, untranslated regions, and promoters; (c) for SNPs in miRNA biogenesis pathway genes, they must have been reported to be significantly associated with the survival of cancer; and (d) for SNPs in pre-miRNA and pri-miRNA, their mature counterparts must have been reported to be implicated in cancer etiology or prognosis (supplemental online Table 1).

### Genotyping

Genomic DNA was extracted from peripheral blood samples collected at the time of diagnosis with the RelaxGene Blood DNA System according to the manufacturer's protocol (Tiangen Biotech, Beijing, People's Republic of China, <http://www.tiangen.com>) and stored at  $-80^{\circ}\text{C}$  until used. All polymorphisms were genotyped by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The PCR primers used to amplify DNA fragments and restriction enzymes applied to digest the PCR products for each SNP are summarized in supplemental online Table 2. The digested products were analyzed using 3% agarose gel electrophoresis.

### Statistical Analysis

The endpoint of the study was overall survival (OS), defined as the time from the date of TCL diagnosis to the date of death by any cause or last follow-up. Allelic distributions of all SNPs were tested for Hardy-Weinberg equilibrium. The Cox proportional hazard model was used to assess the association of SNPs with OS after the assumption of proportionality was met. Hazard ratios (HRs) with 95% confidence intervals (CIs) on the risk of death were estimated adjusting for covariates that might influence survival, including subtype and IPI score. Three different genetic models, including dominant model (comparing homozygous wild-type genotype with variant allele-carrying genotypes), recessive model (comparing wild-type allele-carrying genotypes with homozygous variant genotype), and additive model ( $p$  for trend), were tested and the model that yielded the smallest  $p$  value was considered the best-fitting model and was ultimately applied to the analysis [16]. The Kaplan-Meier method was used to plot the OS curves and the log-rank test was used to compare survival difference between the genotypes. Stratified analyses by TCL subtype (precursor T type vs. mature T type) and IPI score (0–1 vs. 2–3 vs. 4–5) were performed to investigate the association of miRNA-related SNPs with OS in the subsets of TCL patients.

The cumulative effects of SNPs significantly associated ( $p < .05$ ) with TCL survival were assessed by counting the numbers of unfavorable genotypes in each subject and were analyzed using the multivariate Cox proportional hazard model adjusting for subtype and IPI score after the proportionality assumption was satisfied. We performed survival tree analyses with the recursive partitioning method to build a decision tree using the STREE program (<http://c2s2.yale.edu/software/stree>) [17]. The root node of the survival tree included all 220 patients. The log-rank test statistic was used as node-splitting criteria. The recursive procedure continues to produce offspring nodes until no further statistically significant split is obtained. The resulting tree was binary, and each terminal node represented a group of patients with different survival outcomes depending on different genotype combinations. HRs and their 95% CIs for each terminal node were calculated using the multivariate Cox proportional hazard model adjusting for subtype and IPI score after the proportionality assumption was satisfied.

Statistical analyses were carried out using SPSS version 20 (SPSS, Chicago, IL). All tests were two-sided, with a significance level set at  $p < .05$ .

## RESULTS

### Patient Characteristics

Detailed characteristics of the 220 patients are listed in Table 1. The median age at the time of diagnosis was 37 years (range = 6–80), and 151 (68.6%) patients were men. The most common subtype of TCL was natural killer/T-cell lymphoma (25.0%), followed by peripheral T-cell lymphoma not otherwise specified (22.3%), T-lymphoblastic lymphoma/leukemia (20.5%), anaplastic large cell lymphoma (17.7%), angioimmunoblastic T-cell lymphoma (6.8%), subcutaneous panniculitis-like T-cell lymphoma (4.5%), mycosis fungoides (1.4%), enteropathy-type intestinal T-cell lymphoma (0.9%), and Lennert lymphoma (0.9%). One hundred seventeen (54.4%) patients had IPI scores of 0–1, 87

**Table 1.** Patient characteristics and their association with overall survival

| Characteristic                   | All, n (%) | Death, n (%) | HR (95% CI)      | p value           |
|----------------------------------|------------|--------------|------------------|-------------------|
| Median age (yr), n = 220         | 37         | 35           |                  |                   |
| Range                            | 6–80       | 6–80         |                  |                   |
| ≤60                              | 197 (89.5) | 78 (87.6)    | 1.00 (reference) |                   |
| >60                              | 23 (10.5)  | 11 (12.4)    | 1.15 (0.61–2.16) | .667              |
| Gender, n = 220                  |            |              |                  |                   |
| Male                             | 151 (68.6) | 64 (71.9)    | 1.00 (reference) |                   |
| Female                           | 69 (31.4)  | 25 (28.1)    | 0.80 (0.50–1.28) | .351              |
| Smoking status, n = 220          |            |              |                  |                   |
| Nonsmokers                       | 155 (70.5) | 62 (69.7)    | 1.00 (reference) |                   |
| Ever-smokers                     | 65 (29.5)  | 27 (30.3)    | 1.11 (0.71–1.75) | .647              |
| Subtype, n = 220                 |            |              |                  |                   |
| Precursor T type <sup>a</sup>    | 45 (20.5)  | 24 (27.0)    | 1.00 (reference) |                   |
| Mature T type <sup>b</sup>       | 175 (79.5) | 65 (73.0)    | 0.40 (0.24–0.65) | .000214           |
| Stage <sup>c</sup> , n = 220     |            |              |                  |                   |
| I–II                             | 101 (45.9) | 32 (36.0)    | 1.00 (reference) |                   |
| III–IV                           | 119 (54.1) | 57 (64.0)    | 2.18 (1.40–3.37) | .001              |
| Serum LDH <sup>d</sup> , n = 215 |            |              |                  |                   |
| Normal                           | 143 (66.5) | 49 (55.7)    | 1.00 (reference) |                   |
| High                             | 72 (33.5)  | 39 (44.3)    | 2.22 (1.45–3.40) | .000251           |
| ECOG PS, n = 220                 |            |              |                  |                   |
| 0–1                              | 172 (78.2) | 67 (75.3)    | 1.00 (reference) |                   |
| 2–4                              | 48 (21.8)  | 22 (24.7)    | 1.99 (1.21–3.24) | .006              |
| Extranodal site, n = 219         |            |              |                  |                   |
| 0–1                              | 156 (71.2) | 58 (65.2)    | 1.00 (reference) |                   |
| >1                               | 63 (28.8)  | 31 (34.8)    | 1.52 (0.98–2.35) | .062              |
| IPI score <sup>e</sup> , n = 215 |            |              |                  |                   |
| 0–1                              | 117 (54.4) | 40 (45.5)    | 1.00 (reference) | .005 <sup>f</sup> |
| 2–3                              | 87 (40.5)  | 42 (47.7)    | 1.80 (1.16–2.79) | .009              |
| 4–5                              | 11 (5.1)   | 6 (6.8)      | 3.11 (1.31–7.40) | .010              |

<sup>a</sup>Comprised 45 T-lymphoblastic lymphoma/leukemia cases.

<sup>b</sup>Comprised 55 natural killer/T-cell lymphoma cases, 49 peripheral T-cell lymphoma not otherwise specified cases, 39 anaplastic large cell lymphoma cases, 15 angioimmunoblastic T-cell lymphoma cases, 10 subcutaneous panniculitis-like T-cell lymphoma cases, 3 mycosis fungoides cases, 2 enteropathy-type intestinal T-cell lymphoma cases, and 2 Lenert's lymphoma cases.

<sup>c</sup>Defined with Ann Arbor staging system.

<sup>d</sup>Cutoff was 240 IU/L for adults and 270 IU/L for children.

<sup>e</sup>Comprised age, stage, extranodal sites, serum lactic dehydrogenase level, and Eastern Cooperative Oncology Group performance status.

<sup>f</sup>*p*<sub>trend</sub>.

Because of rounding, percentages do not always add to 100.

Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; IPI, International Prognostic Index; LDH, serum lactate dehydrogenase.

(40.5%) had IPI scores of 2–3, and 11 (5.1%) had IPI scores of 4–5. By the time of final analysis on July 1, 2013, 89 (40.5%) patients had died, resulting in a 5-year OS rate of 43.0%.

### Overall Survival by Clinical Characteristics of TCL Patients

We first investigated whether various clinical characteristics had contributed to OS. We stratified patients by age (≤60 years or >60 years), gender, smoking status (nonsmokers or ever-smokers), subtype (precursor T type or mature T type), stage (I–II or III–IV), lactic dehydrogenase (LDH) level (normal or high), Eastern Cooperative Oncology Group (ECOG) performance status (0–1 or 2–4), extranodal site (0–1 or >1), and IPI score (0–1 or 2–3 or 4–5) and compared OS between groups using the univariate Cox proportional hazard model (Table 1).

We found that subtype, individual item of IPI score (including stage, LDH level, and ECOG performance status), and total IPI score significantly affected the survival of patients. The 5-year OS rate was 24.7% for patients with precursor T type and 48.6% for those with mature T type ( $p = .000214$ ). The 5-year OS rates for patients with IPI scores of 0–1, 2–3, and 4–5 were 48.3%, 36.5%, and 16.7%, respectively ( $p = .003$ ).

### Association Between Individual SNPs and TCL Overall Survival

The genotype distributions of the SNPs were in accordance with Hardy-Weinberg equilibrium (Table 2), except for *mir196a 2* rs11614913, which was therefore excluded from further analyses.

**Table 2.** Association of individual single nucleotide polymorphisms (SNPs) with overall survival of T-cell lymphoma patients

| Gene             | SNP        | HWE test | Best-fitting model | Genotype | n   | Death, n (%) | HR <sup>a</sup> (95% CI) | p <sup>a</sup> | FYS (%) | p <sup>b</sup> |
|------------------|------------|----------|--------------------|----------|-----|--------------|--------------------------|----------------|---------|----------------|
| <i>DROSH</i>     | rs10719    | 0.525    | REC                | CC + CT  | 202 | 84 (41.6)    | 1.00 (reference)         |                | 41.5    |                |
|                  |            |          |                    | TT       | 18  | 5 (27.8)     | 0.54 (0.22–1.34)         | .183           | 65.2    | .213           |
|                  | rs6877842  | 0.671    | DOM                | GG       | 195 | 84 (43.1)    | 1.00 (reference)         |                | 36.4    |                |
|                  |            |          |                    | CG       | 25  | 5 (20.0)     | 0.27 (0.11–0.67)         | .005           | 76.1    | .003           |
| <i>DICER</i>     | rs3742330  | 0.903    | REC                | AA + AG  | 200 | 87 (43.5)    | 1.00 (reference)         |                | 38.7    |                |
|                  |            |          |                    | GG       | 20  | 2 (10.0)     | 0.19 (0.05–0.77)         | .020           | 88.4    | .006           |
| <i>GEMIN4</i>    | rs3744741  | 0.397    | DOM                | CC       | 110 | 43 (39.1)    | 1.00 (reference)         |                | 48.7    |                |
|                  |            |          |                    | TT + TC  | 110 | 46 (41.8)    | 1.15 (0.75–1.76)         | .525           | 36.3    | .320           |
| <i>mir146a</i>   | rs2910164  | 0.175    | REC                | CC + CG  | 185 | 71 (38.4)    | 1.00 (reference)         |                | 45.4    |                |
|                  |            |          |                    | GG       | 34  | 18 (52.9)    | 1.49 (0.89–2.51)         | .132           | 31.9    | .157           |
| <i>mir149</i>    | rs2292832  | 0.320    | DOM                | TT       | 106 | 49 (46.2)    | 1.00 (reference)         |                | 31.1    |                |
|                  |            |          |                    | CC + CT  | 114 | 40 (35.1)    | 0.63 (0.41–0.96)         | .031           | 53.7    | .019           |
| <i>mir196a-2</i> | rs11614913 | 0.018    | REC                | CC + CT  | 177 | 73 (41.2)    | 1.00 (reference)         |                | 42.3    |                |
|                  |            |          |                    | TT       | 43  | 16 (37.2)    | 0.91 (0.53–1.58)         | .745           | 43.5    | .585           |
| <i>mir27a</i>    | rs895819   | 0.918    | REC                | AA + AG  | 201 | 79 (39.3)    | 1.00 (reference)         |                | 44.9    |                |
|                  |            |          |                    | GG       | 19  | 10 (52.6)    | 1.69 (0.87–3.31)         | .124           | 31.8    | .208           |
| <i>mir423</i>    | rs6505162  | 0.872    | DOM                | AA       | 130 | 56 (43.1)    | 1.00 (reference)         |                | 40.8    |                |
|                  |            |          |                    | CC + AC  | 90  | 33 (36.7)    | 0.67 (0.43–1.03)         | .065           | 46.8    | .091           |
| <i>mir492</i>    | rs2289030  | 0.964    | DOM                | GG       | 120 | 56 (46.7)    | 1.00 (reference)         |                | 49.7    |                |
|                  |            |          |                    | CC + CG  | 100 | 33 (33.0)    | 0.78 (0.51–1.20)         | .254           | 39.6    | .207           |
| <i>mir499</i>    | rs3746444  | 0.118    | REC                | TT + TC  | 205 | 87 (42.4)    | 1.00 (reference)         |                | 39.8    |                |
|                  |            |          |                    | CC       | 14  | 2 (14.3)     | 0.24 (0.06–0.98)         | .047           | 84.4    | .024           |
| <i>mir124-1</i>  | rs531564   | 0.956    | REC                | CC + CG  | 214 | 86 (40.2)    | 1.00 (reference)         |                | 43.6    |                |
|                  |            |          |                    | GG       | 5   | 2 (40.0)     | 1.61 (0.39–6.57)         | .508           | 50.0    | .452           |
| <i>mir26a-1</i>  | rs7372209  | 0.468    | REC                | TT + TC  | 202 | 79 (39.1)    | 1.00 (reference)         |                | 41.5    |                |
|                  |            |          |                    | CC       | 18  | 10 (55.6)    | 1.18 (0.61–2.29)         | .622           | 53.1    | .558           |

<sup>a</sup>Calculated with multivariate Cox models and adjusted for subtype and International Prognostic Index score.

<sup>b</sup>Based on the log-rank test.

Abbreviations: ADD, additive; CI, confidence interval; DOM, dominant; FYS, 5-year overall survival rate; HR, hazard ratio; HWE, Hardy-Weinberg Equilibrium; REC, recessive.

Overall, four SNPs showed a significant association with OS of patients with TCL using the multivariate Cox proportional hazard model after adjustment for subtype and IPI score (Table 2). Among these four SNPs, the dominant model best fit the data for *DROSHA* rs6877842 and *mir149* rs2292832; the recessive model best fit the data for *DICER* rs3742330 and *mir499* rs3746444. Patients with the variant allele-carrying genotypes of *DROSHA* rs6877842 and *mir149* rs2292832 had a significantly increased OS compared with those carrying the homozygous wild-type genotype (HR, 0.27; 95% CI, 0.11–0.67;  $p = .005$ ; and HR, 0.63; 95% CI, 0.41–0.96;  $p = .031$ , respectively). The same was true for the patients with the homozygous variant genotype of *DICER* rs3742330 and *mir499* rs3746444 compared with those carrying the wild-type allele-carrying genotypes (HR, 0.19; 95% CI, 0.05–0.77;  $p = .020$ ; and HR, 0.24; 95% CI, 0.06–0.98;  $p = .047$ , respectively).

To investigate the effects of the four SNPs on OS in subsets of patients with TCL, we performed stratified analyses based on TCL subtype (precursor T type vs. mature T type) and IPI score (0–1 vs. 2–3 vs. 4–5). The variant allele-carrying genotypes of *DROSHA* rs6877842 and *mir149* rs2292832 and the homozygous variant genotype of *DICER* rs3742330 and *mir499*

rs3746444 were also associated with improved OS in patients with mature T type but not in patients with precursor T type (supplemental online Table 3). In a stratified analysis by IPI score, we found that the association with better OS remained significant for *DROSHA* rs6877842, *DICER* rs4742330, and *mir499* rs3746444 in patients with an IPI score of 0–1 but not in patients with IPI scores of 2–3 and 4–5 (supplemental online Table 4).

### Cumulative Effects of the Unfavorable Genotypes on Overall Survival

We further evaluated the cumulative effects of SNPs on TCL survival by counting the unfavorable genotypes of the four SNPs identified. We found that compared with patients with zero, one, or two unfavorable genotypes, those with three unfavorable genotypes were at a 2.53-fold (95% CI, 1.06–6.00;  $p = .036$ ) increased risk of death, and the risk further increased to 5.31-fold (95% CI, 2.24–12.57;  $p = .00015$ ) for those with all four unfavorable genotypes ( $p_{\text{trend}} = .00004$ ) (Table 3). The 5-year OS rates were 78.0%, 49.2%, and 16.9% for the above three groups of patients, respectively ( $p = .000005$ ) (Fig. 1).

**Table 3.** Cumulative effects of unfavorable genotypes on T-cell lymphoma survival

| Number of unfavorable <sup>a</sup> genotypes | n   | Death, n (%) | FYS (%) | HR <sup>b</sup> (95% CI) | p <sup>b</sup>      |
|--|-----|--------------|---------|--------------------------|---------------------|
| 0–2  | 31  | 6 (19.4)     | 78.0    | 1.00 (reference)         | .00004 <sup>c</sup> |
| 3  | 107 | 37 (34.6)    | 49.2    | 2.53 (1.06–6.00)         | .036                |
| 4  | 82  | 46 (56.1)    | 16.9    | 5.31 (2.24–12.57)        | .00015              |

<sup>a</sup>Definition of unfavorable genotypes: *DROSHA* rs6877842 WW, *DICER* rs3742330 WW/MM, *mir149* rs2292832 WW, *mir499* rs3746444 WW/MM. W, wild-type allele; M, variant allele.

<sup>b</sup>Calculated with multivariate Cox models and adjusted for subtype and International Prognostic Index score.

<sup>c</sup>p<sub>trend</sub>.

Abbreviations: CI, confidence interval; FYS, 5-year overall survival rate; HR, hazard ratio.

### Survival Tree Analysis

To explore potential high-order interactions between SNPs and to define subgroups that have distinct survival prospects, we performed a survival tree analysis using the four SNPs identified in the individual SNP analysis. The tree structure resulted in three terminal nodes. The top splitting factor was *DROSHA* rs6877842, followed by *mir149* rs2292832 (Fig. 2A). Taking terminal node 1 (patients with the homozygous wild-type genotypes of both rs6877842 and rs2292832) as the reference group, the HR for terminal node 2 (patients with the homozygous wild-type genotype of rs6877842 and at least one variant allele of rs2292832) was 0.58 (95% CI, 0.37–0.91;  $p = .017$ ), and the HR for terminal node 3 (patients with the heterozygous genotype of rs6877842) was 0.20 (95% CI, 0.08–0.51;  $p = .001$ ) (Fig. 2A). The 5-year OS rates were 21.9% for patients in terminal node 1, 49.4% for patients in terminal node 2, and 76.1% for patients in terminal node 3 ( $p = .000316$ ) (Fig. 2B).

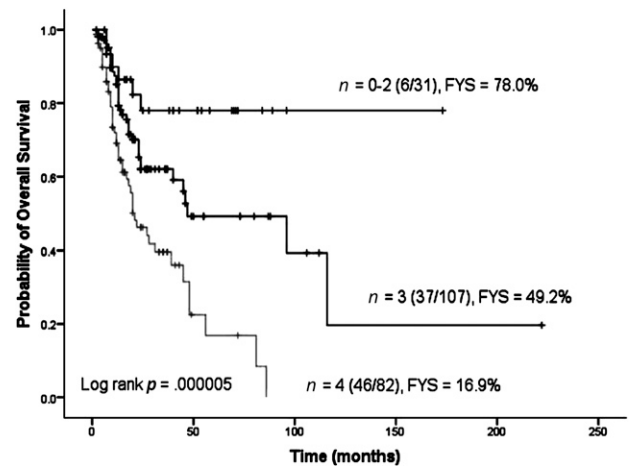
### DISCUSSION

To apply individualized therapy for TCL, we need to predict the prognosis of each patient according to his/her pretherapeutic features [18]. It is increasingly suggested that SNPs, the most common genetic variants in humans, as a whole are key enablers [19]. MiRNA-related genes have been reported to be associated with biological features of T-cells and clinical outcomes of TCL [10, 20], but whether SNPs in these genes are associated with survival of TCL is still unclear.

Therefore, we carefully selected 13 SNPs in miRNA-related genes based on prespecified criteria and evaluated their roles in the prognosis of patients with TCL. We found that *DROSHA* rs6877842, *DICER* rs3742330, *mir149* rs2292832, and *mir499* rs3746444 were associated with the survival of patients. Furthermore, we observed a dose-dependent cumulative effect of these SNPs on the survival of patients and a higher order interaction between these SNPs.

For individual SNP analysis, *DROSHA* rs6877842 showed the most statistically significant association with TCL survival. *DROSHA* initiates the miRNA biogenesis in the nucleus [21]. Previous studies have demonstrated that altered expression of *DROSHA* may alter the miRNA expression profile associated with survival of cancer patients [22, 23]. SNP rs6877842 is located in the promoter of *DROSHA* and may affect the expression of *DROSHA* through affecting the binding of transcription factor to this region; hence, it may be associated with the survival of patients with TCL.

Besides the newly identified significant SNP rs6877842 in *DROSHA*, we also confirmed the previously reported association of *DICER* rs3742330 with survival of patients with TCL [14]

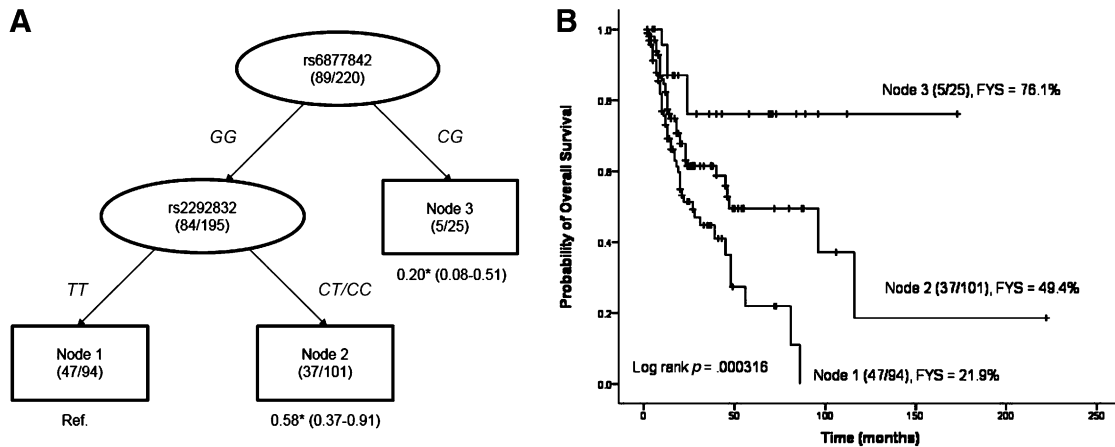


**Figure 1.** Overall survival of patients with T-cell lymphoma according to different numbers of unfavorable genotypes. Abbreviations: FYS, 5-year overall survival rate;  $n$ , number of unfavorable genotypes.

after more patients were tested and the follow-up period was extended. *DICER* is essential for the production of mature miRNAs through cleaving the double-strand pre-miRNA, and silencing of *DICER* in cells can reduce pre-miRNA and mature miRNA sequences [24]. SNP rs3742330 is located in the 3'-UTR, which may affect the expression of *DICER* through changing the binding capacity of regulatory miRNAs [25, 26], thus affecting the survival rate of TCL.

SNPs in the pri- or pre-miRNA regions were also evaluated, among which *mir149* rs2292832 and *mir499* rs3746444 were demonstrated to be significantly associated with the survival of patients with TCL. *Mir149* rs2292832 and *mir499* rs3746444 have been previously reported to be significantly associated with the survival of patients with other cancers [27–30]. The predicted target protein TP63 of *mir149* has been reported to be associated with the survival of patients with TCL [31]. The potential targeted protein NOTCH1 of *mir499* was a biomarker for predicting the survival of TCL patients [32]. These analyses suggested that it is highly likely that the miRNAs identified in this study are actively involved in affecting the survival of patients with TCL.

Histological subtype and IPI score are major clinical characteristics that are associated with the survival of patients with TCL as demonstrated in this and previous studies [2, 33]. We performed stratified analyses according to subtype or IPI score to investigate the role of identified SNPs in different subsets of patients. When the patients were divided by subtype, the four SNPs remained significantly associated with



**Figure 2.** Survivability defined by high-order interactions between SNPs. **(A):** Survival tree analysis of survival of patients with T-cell lymphoma. **(B):** Overall survival according to different nodes identified by survival tree analysis. The number under each node represents the hazard ratio with 95% confidence interval in parenthesis. \*,  $p < .05$ . Abbreviation: C, cytosine; FYS, 5-year overall survival rate; G, guanine; T, thymine.

survival in patients with mature T type, but not in patients with precursor T type. When patients were divided by IPI score, three SNPs remained significantly associated with survival in patients with IPI scores of 0–1, but not in patients with IPI scores of 2–3 or 4–5. This discordance between different subsets of patients may be due to the limited number of patients in the study. Another possible explanation is that the poor prognostic role of unfavorable genotypes is inferior to that of subtype or IPI score; thus, the effect of these SNPs can be prominent only in patients with better potential prognosis.

Prognosis of patients is usually predicted by combinations of multiple factors. In order to explore the combined effects of these miRNA-related SNPs on the survival of TCL, we performed cumulative effect analysis by counting the number of unfavorable genotypes. Using the Cox proportional hazard model, we identified a trend of poor survival with an increasing number of unfavorable genotypes in a dose-dependent manner.

Cox proportional hazard models are used to investigate the impact of certain factors on prognosis, and the survival tree method is to define subgroups that have distinct survival prospects. The graphical output of the latter facilitates the visualization of prognostic groups, reflecting multimarker interactions. This method is usually used in a complementary fashion with the Cox proportional model [34]. In our survival tree analysis, we found a higher order interaction between these SNPs and identified that the subgroup of patients with the homozygous wild-type genotypes of both rs6877842 and rs2292832 had the lowest survival probability, whereas those with the heterozygous genotype of rs6877842 had the highest probability of survival. These results suggested that the clinical progression or remission of TCL may be a polygenic process and

the pathway-based multiple gene approach has high predictive power.

## CONCLUSION

miRNA-related SNPs *DROSHA* rs6877842, *DICER* rs3742330, *mir149* rs2292832, and *mir499* rs3746444 are associated with the survival of TCL patients and can be used to predict the prognosis of TCL individually and collectively. Nonetheless, these results are to be validated in larger cohorts of patients, and the biological functions of these SNPs remain to be explored.

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## DISCLOSURES

The authors indicated no financial relationships.

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