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Original contribution

miR-31 and miR-17-5p levels change during transformation of follicular lymphoma☆,☆☆

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Summary The 30% of patients whose indolent follicular lymphoma transforms to aggressive diffuse large B-cell lymphoma (DLBCL) have poor survival. Reliable predictors of follicular B-cell lymphoma transformation to DLBCL are lacking, and diagnosis of those that will progress is challenging. MicroRNA, which regulates gene expression, has critical functions in the growth and progression of many cancers and contributes to the pathogenesis of lymphoma. Using 5 paired samples from patients who presented with follicular lymphoma and progressed to DLBCL, we identified specific microRNA differentially expressed between the two. Specifically, miR-17-5p levels were low in follicular lymphoma and increased as the disease transformed. In contrast, miR-31 expression was high in follicular lymphoma and decreased as the lymphoma progressed. These results were confirmed in additional unpaired cases of low-grade follicular lymphoma (n = 13) and high-grade follicular lymphoma grade 3 or DLBCL (n = 17). Loss of miR-31 expression in DLBCL was not due to deletion of the locus. Changes in miR-17-5p and miR-31 were not correlated with immunophenotype, genetics, or status of the *MYC* oncogene. However, increased miR-17-5p expression did significantly correlate with increased expression of p53 protein, which is indicative of mutant *TP53*. Two pro-proliferative genes, *E2F2* and *PI3KC2A*, were identified as direct messenger RNA targets of miR-31, suggesting that these may contribute to follicular lymphoma transformation. Our results indicate that changes in miR-31 and miR-17-5p reflect the transformation of follicular lymphoma to an aggressive large B-cell lymphoma and may, along with their targets, be viable markers for this process.

1. Introduction

Follicular lymphoma (FL), a low-grade non-Hodgkin B-cell lymphoma, accounts for 20% of all lymphomas [1]. Translocation of the antiapoptotic gene *BCL2* to the immunoglobulin heavy chain locus *IGH*, t(14:18), occurs in 85% of cases [2]. Additionally, chromosomal rearrangements of the transcriptional repressor *BCL6* are present in 5% to 15% of FL [3]. A 28% rate of transformation at 10 years of low-grade FL (World Health Organization [WHO] grades FL1 and FL2) into a more aggressive large cell

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lymphoma, either WHO grade 3 FL (FL3) or diffuse large B-cell lymphoma (DLBCL), was reported with a cumulative rate of transformation of 3% per year [4]. Follicular lymphoma grade 3 is characterized by neoplastic follicles that contain greater than 15 large transformed cells per high-power field (FL3A) or are composed solely of large transformed cells (FL3B). DLBCL consists of sheets of large transformed B cells with loss of the follicular growth pattern [1]. Both of these transformed lymphomas progress rapidly, require aggressive chemotherapy, and have poor survival rates [4]. Multiple pathways of transformation to DLBCL have been proposed [5-9], yet what distinguishes one FL that transforms from one that does not progress still remains unclear. Elucidation of mechanisms of transformation may suggest novel therapeutic targets to prevent or arrest transformation. In addition, early markers predicting the likelihood of FL transformation would help to initiate aggressive therapy before the patient developed widespread involvement by FL3 or DLBCL.

MicroRNA (miRNA) are small noncoding RNA that regulate gene expression posttranscriptionally by binding to the 3′ untranslated region (UTR) of messenger RNA (mRNA), inhibiting translation and/or facilitating mRNA degradation. They are critical modulators of many biological processes, such as cell growth, differentiation, and apoptosis, linked to the development and progression of many tumor types, including lymphomas [10]. The *miR-17-92* cluster is induced by the oncogene *MYC*, which drives numerous malignancies including Burkitt lymphoma and many DLBCL [11,12]. Overexpression of the *miR17-92* polycistron specifically in B cells induces B-cell lymphoma development, leading to its oncomiR label [13,14]. Other miRNA are thought to be tumor suppressor miRNA because their expression inhibits tumor development or progression [10]. Still other miRNA, such as miR-31, have been linked to both oncogenic and tumor suppressive functions in different cancers [15].

There have been attempts to distinguish different lymphoma subtypes by the miRNA they express, which has led to miRNA signatures characterizing FL and DLBCL [5,16]. Here, we compare miRNA expression from paired samples of low-grade FL and aggressive FL3 or DLBCL biopsied when the patient progressed. Our analyses of these lymphomas identify miR-17-5p and miR-31 as differentially regulated miRNA in FL compared to DLBCL. Our data show that as FL progresses to aggressive large B-cell lymphoma, miR-17-5p increases and miR-31 decreases, establishing these 2 miRNA as markers of FL progression.

2. Materials and methods

2.1. Patient selection

Vanderbilt University Medical Center hematopathology/flow cytometry databases from 2000 to 2014 were searched for patients with a diagnosis of FL or DLBCL with institutional review board approval. Diagnostic slides from those patients with biopsies of both low-grade FL and FL3 or DLBCL were reviewed by 2 hematopathologists (M. A. T. and S. M.-T.). Confirmation of FL was based upon morphology and immunohistochemical or flow cytometric positivity for CD10, immunohistochemical positivity for BCL6, or cytogenetic demonstration of the t(14;18) *IGH/BCL2* translocation. Only DLBCL with CD10 positivity was included, and DLBCL with cytogenetic documentation of *MYC* translocation was excluded. Of the 13 nonmatched DLBCL, 5 had a history of FL before the DLBCL or residual regions of FL in the current biopsy (Table). Four additional cases had *IGH/BCL2* translocation by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR), suggesting an FL origin. Of the 13 nonmatched FL1/FL2 patients, 10 had 1- to 3-year follow-up available, and of those, 3 progressed to FL3/DLBCL (Table). Four of the FL1/FL2 had a previous FL3 or DLBCL. The paired samples from 1 patient who demonstrated the most uniform histology of FL1 and DLBCL was selected for the array analysis. The WHO criteria for grade 1 to 3 FLs were used [1]. Pertinent patient information is listed in the Table. The nonneoplastic normal lymph node samples were diagnosed as "reactive follicular hyperplasia."

2.2. miRNA isolation and expression analysis

Total RNA was isolated from paraffin sections of archival lymphoma biopsies (RecoverAll; Ambion, Austin, TX, USA). For cell lines, miRNA was isolated with Trizol (Life Technologies, Carlsbad, CA, USA) as we previously reported [17]. TaqMan microRNA low-density arrays (TLDA; 365 human miRNAs and 2 small nucleolar RNA (snoRNA); Applied Biosystems, Foster City, CA, USA) were performed in duplicate by the Vanderbilt Technologies for Advanced Genomics core facility on a FL1 and a DLBCL biopsied at separate times from a single patient (1A and 1B in the Table). Data were analyzed with RQ manager software, and the individual quantitative real-time polymerase chain reactions (qRT-PCRs) were reviewed to assess the quality of each reaction. qRT-PCR for specific miRNA was performed using the Applied Biosystems TaqMan microRNA assay as previously reported [17]. Small RNA controls, RNU24 for patient samples and RNU6B for cell lines, were used to normalize the data. Where indicated, miRNA data were normalized to results from normal lymph node. Unpaired and paired *t* tests were performed to determine significance.

2.3. Fluorescence in situ hybridization

FISH probes for the *MIR31* locus (chromosome 9p21) and the *CDKN2A* locus (chromosome 9p22) were prepared by labeling BAC-PAC clones RP11-354P17 and RP11-149I2, respectively (Children's Hospital Oakland Research Center). DNA was labeled by nick translation (Abbott Laboratories, Des Plaines, IL) using Spectrum Green