Correlation between the overexpression of Yin Yang 1 and the expression levels of miRNAs in Burkitt's lymphoma: A computational study

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Abstract. A growing number of studies have highlighted the role of microRNAs (miRNAs or miRs) in the development and progression of cancer. In particular, the aberrant expression of cancer-related proteins, such as oncogenes and tumor suppressors has been shown to correlate with the modulation of the expression of specific miRNAs. In the present study, we aimed to determine which downregulated miRNAs may be involved in modulating the expression of the oncogenic transcription factor, Yin Yang 1 (YY1). YY1 has been reported to be overexpressed in several malignancies and our previous studies have highlighted the significant correlation between the levels of YY1 and aggressive behavior in non-Hodgkin's lymphoma (NHL). A total of 57 miRNAs that are potentially capable of targeting YY1 was identified through *in silico* approaches. The search of publicly available NHL datasets, including paired mRNA and miRNA data (GSE23026) highlighted a significant correlation (Pearson's correlation, r>0.5) between the expression levels of YY1 and the expression levels of a limited set of miRNAs, including miR-363, miR-200a, miR-23b, miR-15a and miR-15b. Intriguingly, both hsa-miR-363 and hsa-miR-200a belong to the top 20 miRNAs that were found to be downregulated in Burkitt's lymphoma (BL) tissue compared to normal tissue. Although further validation studies are warranted, the identification of these two miRNAs associated with the upregulation of YY1 in BL may provide further insight into the pathogenesis of this tumor and may contribute to more personalized and targeted treatment approaches for patients with this disease.

Introduction

MicroRNAs (miRNAs or miRs) are small non-coding RNAs, comprising 19-25 nucleotides, that reduce the abundance and translational efficiency of mRNAs and play a major role in regulatory networks, influencing diverse biological processes through the effects of individual miRNAs on the translation of multiple mRNAs (1,2). Determining the roles of individual miRNAs in cellular regulatory processes poses a major challenge with the function of the majority of miRNAs that are currently unknown; even for relatively well-studied miRNAs, only a handful of targets have been rigorously characterized and these may differ by tissue type.

miRNAs post-transcriptionally regulate the expression of thousands of genes, including key genes in cell differentiation and cancer pathogenesis (3). A growing number of studies have highlighted the role of miRNAs in the development and progression of cancer (4,5). In particular, the aberrant expression of cancer-related proteins, such as oncogenes and tumor suppressors has been shown to correlate with the modulation of the expression specific miRNAs.

Non-Hodgkin's lymphomas (NHLs) are a heterogeneous group of malignancies of the lymphoid system. NHLs are classified based on cytology, immunophenotype and both genetic and clinical characteristics (6). Genetic aberration and molecular changes are used for a more appropriate diagnostic profile. The most commonly associated genetic abnormality in NHL is the translocation of t(14;18)(q32;q21) (7), mostly detected in low-grade lymphomas, such as follicular lymphoma; while molecular rearrangements involving Bcl-6 or c-Myc are frequently observed in diffuse large B-cell lymphoma (DLBCL)

and Burkitt's lymphoma (BL), respectively. Currently, a significant percentage of patients with NHL are successfully cured; however, a fraction of these patients may relapse following treatment due to the development of chemoresitance (8,9).

It has been demonstrated that the transcription factor, Yin Yang 1 (YY1), is involved in the regulation of resistance to chemotherapy and immunotherapy in NHL cell lines. In addition, the regulation of Fas resistance by nuclear factor (NF)-κB is mediated via YY1 expression and activity (10). YY1 is known to play a critical role in normal biological processes, such as embryogenesis, differentiation, replication and cell proliferation. YY1 exerts its effects on genes involved in these processes through its ability to initiate, activate, or repress transcription, depending upon the context in which it binds (11).

It has also been reported that YY1 is overexpressed in several malignancies, including B-cell NHL (12,13), and this overexpression is associated with aggressive behaviour in NHL (14). Based on these findings, in the current study, miRNAs potentially capable of targeting and modulating YY1 were identified through *in silico* approaches.

Data collection methods

In order to identify putative miRNAs that target YY1 mRNA, several publicly available miRNA target prediction tools were used, including DIANA-microT (15), miRanda (16), PicTar (17) and TargetScanS (18). Publicly available Gene Expression Omnibus datasets of NHL specimens with the relative normal counterparts were analyzed. Only datasets with both mRNA and miRNA expression levels were considered for the analysis. According to these criteria, the dataset GSE23026 was used in our study (19). This dataset includes 12 samples of BL, 5 tonsil specimens, 7 lymph node specimens and 4 samples of CD19+CD10+ B-cells.

Statistical analysis. The differential analysis of YY1 expression levels was performed using the ANOVA test. Correlation analysis between the miRNA expression and YY1 expression levels was performed using the Pearson correlation test.

Results

The analysis using the Diana-microT, miRanda, PicTar, TargetScanS tools revealed a total of 57 predicted miRNAs capable of targeting YY1 (Fig. 1).

The search of publicly available NHL datasets, including paired mRNA and miRNA data (GSE23026) highlighted a significant negative correlation (Pearson correlation coefficient ≤-0.5) between the YY1 expression levels and the expression of a limited set of miRNAs, including miR-363, miR-200a, miR-23b, miR-15a and miR-15b among the 57 predicted miRNAs capable of targeting YY1 (Table I).

As was expected, the transcript expression levels of YY1 were found to be significantly higher in the BL samples compared to the normal tissue samples (mean value expression for BL=0.458, tonsil=-0.248, lymph node=0.049 and CD19+CD10+ B-cells=0.197; ANOVA, P=0.007) (Fig. 2). Intriguingly, both hsa-miR-363 and hsa-miR-200a belong to the top 20 miRNAs that were found to be downregulated in the BL samples compared to their normal counterparts (Table II).

Table I. Pearson correlation analysis between microRNAs targeting YY1 and YY1 expression levels.

miRNA	Pearson correlation (r≤-0.5)	miRNA ID
[a]hsa-miR-363	-0.611	A_25_P00010953
[a]hsa-miR-200a	-0.608	A_25_P00010209
hsa-miR-23b	-0.577	A_25_P00010881
hsa-miR-200a	-0.576	A_25_P00010208
hsa-miR-15a	-0.552	A_25_P00010467
[a]hsa-miR-363	-0.549	A_25_P00010954
hsa-miR-15b	-0.517	A_25_P00011101

[a]MicroRNAs (miRNAs or miRs) belonging to the top 20 miRNAs that were found to be differentially expressed in Burkitt's lymphoma samples compared to the normal tissue samples. YY1, Yin Yang 1.
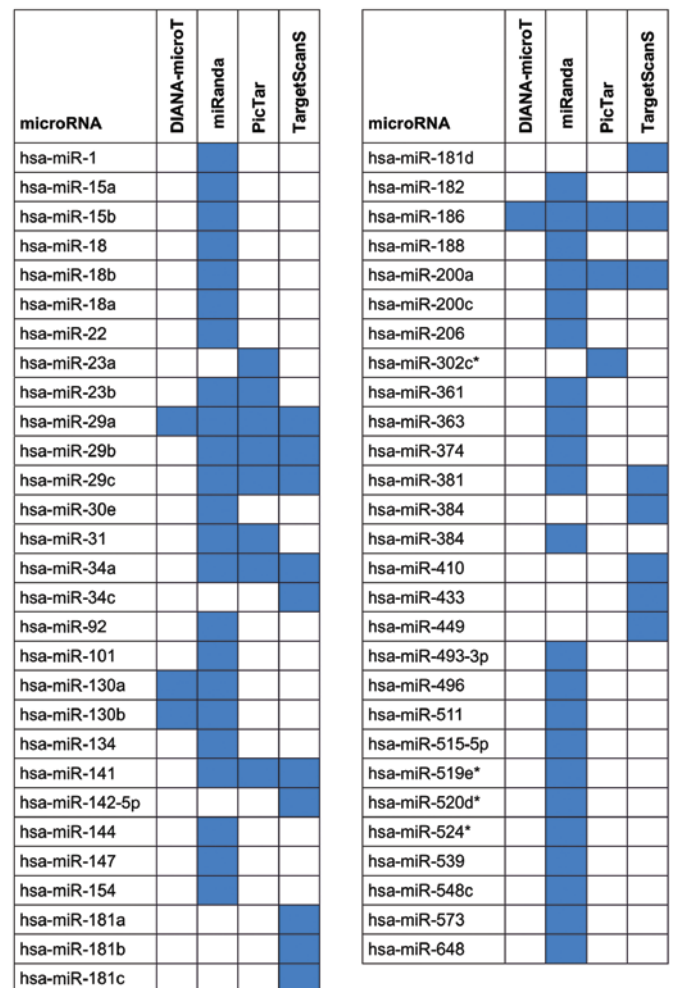

Figure 1. Predicted miRNAs capable of targeting YY1 identified using the Diana-microT, miRanda, PicTar and TargetScanS tools. The tools that recognized each predicted miRNA are indicated by the blue-colored boxes.

Discussion

A growing body of evidence has indicated that deregulated miRNA expression is observed in a variety of human diseases,
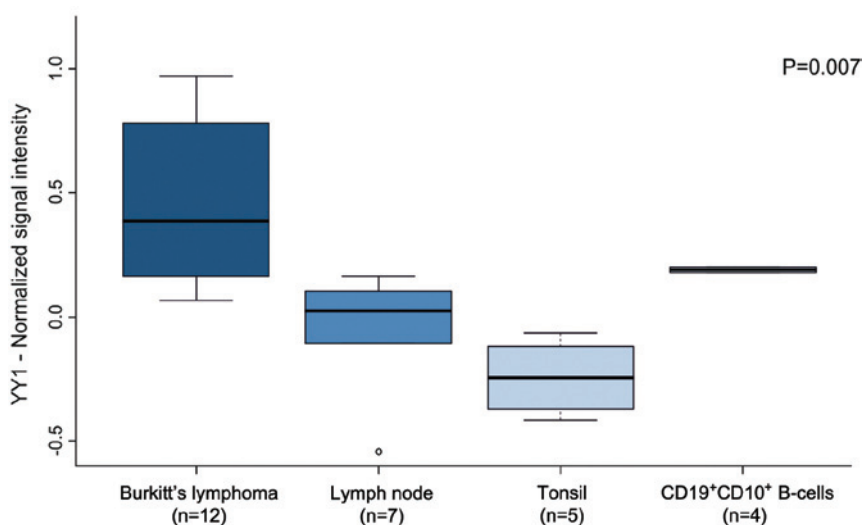
Figure 2. Yin Yang 1 transcript levels in Burkitt's lymphoma (GSE23026) are reported as boxed quartiles (median, 25th, and 75th percentile) and whiskers (minimum and maximum). The differences between tumor and normal samples are compared by the ANOVA test.

Table II. Top 20 microRNAs downregulated in Burkitt's lymphoma samples compared to normal tissue samples.

miRNA	P-value	FC (log)	miRNA ID
hsa-miR-150	2.32E-04	-3.991	A_25_P00010490
hsa-miR-150	3.13E-04	-3.869	A_25_P00010489
hsa-miR-363	1.71E-04	-3.745	A_25_P00010954
hsa-miR-99a	1.10E-06	-3.686	A_25_P00010471
hsa-miR-10b_v9.1	5.03E-05	-3.657	A_25_P00010136
hsa-miR-10b_v9.1	3.53E-05	-3.607	A_25_P00010135
hsa-miR-139_v9.1	2.73E-05	-3.594	A_25_P00010349
hsa-miR-363	9.85E-05	-3.547	A_25_P00010953
hsa-let-7g_v9.1	7.09E-06	-3.431	A_25_P00010306
hsa-miR-31_v9.1	6.93E-05	-3.426	A_25_P00010510
hsa-let-7g_v9.1	5.21E-06	-3.404	A_25_P00010305
hsa-miR-200b_v9.1	1.01E-03	-3.389	A_25_P00010908
hsa-let-7a	7.41E-07	-3.37	A_25_P00011584
hsa-let-7f	1.59E-06	-3.335	A_25_P00010088
hsa-miR-195	5.00E-06	-3.25	A_25_P00010769
hsa-miR-133b	7.41E-04	-3.233	A_25_P00010963
hsa-miR-195	9.00E-06	-3.198	A_25_P00010770
hsa-let-7e_v9.1	1.04E-06	-3.192	A_25_P00010280
hsa-miR-200a	8.78E-04	-3.184	A_25_P00010209
hsa-let-7e_v9.1	6.81E-07	-3.181	A_25_P00010279

Analysis of microRNAs (miRNAs or miRs) was performed using GEO2R tools. FC, fold change.

including lymphomas, suggesting an important role in their pathogenesis (20,21). The data from several experimental studies have supported an important role for miRNAs in lymphomagenesis through systematic miRNA profiling in lymphoma samples using a variety of miRNA expression platforms (22-24).

YY1 is an activator and a repressor transcription factor belonging to the Zinc-finger proteins (25). It is a positive regulator of several oncogenes, such as c-Fos (26), c-Myc (27) and Erb-B2 receptor tyrosine kinase 2 (ERBB2) (28,29). However, it is also a negative regulator of a fraction of tumor suppressor genes, including p27 (30), p16 (31), p73 (32) and p53 (33). YY1 plays a role in the malignant transformation of several diseases, including B-cell lymphomas. Based on these findings, the aim of the present study was to identify, through *in silico* approaches, which miRNAs are potentially capable of targeting and modulating YY1.

Computational evaluation is an appropriate approach for an initial screening to identify deregulated miRNAs. Based on our results, we speculated that YY1 overexpression may be caused by the downregulation of both hsa-miR-363 and hsa-miR-200a, at least in BL. In fact, the overexpression of YY1 negatively correlated with the expression of hsa-miR-363 and hsa-miR-200a. In turn, the expression levels of hsa-miR-363 and hsa-miR-200a were lower in the BL samples when compared with the normal tissue samples. The increased transcript levels of YY1 in the BL samples is in agreement with the observations of a previous study in which the expression of YY1 was found to be associated with high-grade lymphomas, suggesting that it is linked with post-germinal center transit, where genetic alterations may occur, such as Bcl-6 mutations and/or Bcl-6 translocations (34). These findings may provide further insight into the pathogenesis of BL, and YY1 may thus be considered as a molecular signature of aggressiveness in high-grade lymphomas.

Notably, it has been previously reported that the overexpression of YY1 may promote p53 degradation or inhibit its transcriptional activity (35). Furthermore, the inhibition of YY1 in cancer cells, which lack functional p53, also sensitizes them to cell death in response to apoptotic stimuli (36,37). Although p53 is seldom mutated in NHL, the p53 pathway has been reported to be attenuated in these tumors (38). Among the targets of p53, miR-34a has been found to be repressed as a consequence of promoter methylation (39). These findings

support the notion that YY1 may directly control the expression of genes involved in the apoptotic machinery independently of the p53 protein. However, certain drug-resistant hematopoietic disorders that exhibit wild-type p53 may benefit from targeted therapy (40).

In agreement with our results, the loss of miR-200a expression in primary cutaneous melanoma is considered as an additional indicator of aggressiveness, as it was associated with increased thickness and disease progression (41).

Low levels of miR-200a have also been observed in mucosa-associated lymphoid tissue lymphoma (MALT) lymphoma of the conjunctiva. It was also found that cyclin E2 is a target of miR-200a (42). Accordingly, the putative role of YY1 in tumorigenesis is sustained by its interaction with cell cycle regulation. The downregulated expression of miR-363 can precisely differentiate the high-grade lymphomas into ABC or GCB subtypes, and its expression has been shown to correlate with the clinical outcome (43). Similarly, the downregulation of miR-363 has also been detected in ovarian cancer and has been shown to be associated with the drug-resistance phenomenon (44).

Although further validation studies are warranted, the results of the present study indicate that the negative correlation between miR-200a and miR-363 and YY1 expression, which is observed in BL, may provide further insight into the pathogenesis of BL and may have implications on treatment strategies. Furthermore, our data suggest that miRNA expression profiling provides a useful tool for a better understanding of cancer development and progression and for the identification of molecular predictors of response and outcome.

Acknowledgements

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