MIRNAs and their target genes in B cell lymphomas
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Chapter 2

miRNA profiling of B-cell subsets: specific miRNA profile for germinal center B-cells with variation between centroblasts and centrocytes

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Abstract
MicroRNAs (miRNAs) are an important class of small RNAs that regulate gene expression at the post-transcriptional level. It has become evident that miRNAs are involved in hematopoiesis and that deregulation of miRNAs may give rise to hematopoietic malignancies. The aim of our study was to establish miRNA profiles of naive, germinal center (GC) and memory B-cells and validate their expression patterns in normal lymphoid tissues. Quantitative (q)RT-PCR profiling revealed that several miRNAs were elevated in GC B-cells, including miR-17-5p, miR-106a and miR-181b. One of the most abundant miRNAs in all three B-cell subsets analyzed was miR-150 with a more than 10-fold lower level in germinal center B-cell as compared to the other two subsets. MiRNA in situ hybridization (ISH) in tonsil tissue sections confirmed findings from the profiling work. Interestingly, gradual decrease of miR-17-5p, miR-106a, and miR-181b staining intensity from the dark to the light zone was observed in GC. A strong cytoplasmic staining of miR-150 was observed in a minority of the centroblasts in the dark zone of the GC. Inverse staining pattern of miR-150 against c-Myb and Survivin was observed in tonsil tissue sections, suggesting possible targeting of these genes by miR-150. In line with this, experimental induction of miR-150 lead to reduced c-Myb, Survivin and Foxp1 expression levels in the Burkitt lymphoma cell line, DG75. In conclusion, miRNA profiles of naive, GC and memory B-cells were established and validated by miRNA ISH. Within the GC cells a marked difference was observed between the light and the dark zone.
Introduction

MiRNAs are a class of small RNAs, of 19-23 nucleotides that were discovered less than two decades ago (1;2). When incorporated into Argonaute (Ago) proteins to form the RNA induced silencing complex (RISC), miRNAs can negatively regulate genes at the post-transcriptional level by either triggering translational repression or direct cleavage of mRNAs (3). MiRNA expression can be highly tissue specific (4) and are known to regulate many cellular processes including proliferation, apoptosis, cell cycle, differentiation and hematopoiesis (5-8).

The distinct and dynamic expression pattern of miRNAs during hematopoiesis reflects the importance of this class of small RNAs in determining the various states of differentiation of hematopoietic cells (9-12). Alteration in expression level of merely one miRNA in hematopoietic stem cells can skew the differentiation process towards a specific hematopoietic cell type (13). For this reason, it was assumed that aberrant expression of miRNAs could be involved in the development of hematopoietic malignancies. Indeed, the specific involvement of a number of miRNAs not only in hematopoietic development but also lymphomagenesis has been the subject of a growing number of studies in the past few years.

C-Myb, a transcription factor known to play a key role in B-cell maturation, has been shown to be a direct target of miR-150 (14). Ectopic expression of miR-150 in hematopoietic stem cells leads to blockage of the transition of pre-B to pro-B stage (15). The PU.1 transcription factor, essential in hematopoietic lineage development, and activation-induced cytidine deaminase (AID), a protein that is important in class switch recombination of the immunoglobulin locus, have been demonstrated to be targets of miR-155 (16;17). As a consequence of miR-155 down regulation, inefficient germinal center response causing production of less IgG\(_1\) positive cells was observed. Li et al. demonstrated that miR-181a can fine tune T-cell sensitivity during the maturation process by directly targeting SHP-2, PTPN22, DUSP5, and DUSP6, four components of the T-cell receptor signaling pathway (18). In parallel with the discoveries of important hematopoietic miRNAs, it has become evident that miRNAs are involved in the pathogenesis of leukemia and lymphoma. For example, miR-21, miR-92, miR-150, miR-155 and miR-222 are reported to be deregulated in
chronic lymphocytic leukemia (CLL) (19;20). Over-expression of miR-155 as well as the miR-17-92 cluster has been documented for several B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL) (21-23). In a transgenic mouse model, ectopic expression of miR-155 leads to lymphoblastic leukemia/high grade lymphoma (24).

Recently, several studies have been carried out to establish miRNA profiles of hematopoietic cells (25;26), but none of them are confirmed by in situ hybridization (ISH). The aim of our study was to characterize the miRNA expression profile of normal B-cell subsets, which includes naive, germinal center (GC) and memory B-cells. Since gene expression analysis identified only limited differences between centroblasts and centrocytes (27;28), both cell types were grouped as GC B-cells in our study. By comparing these profiles, GC B-cell specific miRNA expression patterns were defined. Findings from the profiling work were validated with miRNA in situ hybridization (ISH) technique and expressions of three miR-150 targets were examined.

Materials and methods

Tissue sections

Tissue samples of hyperplastic tonsils removed from children with chronic tonsillitis were obtained from the department of Ear Nose Throat, University Medicine Center Groningen. All protocols for obtaining and studying human tissues and cells were approved by the institution's review board for human subject research.

FACS sorted normal B-cell subsets

Normal B-cell subsets were sorted by FACS from three different tonsil samples, essentially as described previously (29). Briefly, mononuclear cells were isolated by Ficoll-Isopaque density gradient centrifugation. The collected cell suspension was stained with FITC-conjugated anti-human IgD, PE-conjugated anti-human CD19 (both from Dako, Glostrup, Denmark), and allophycocyanin-conjugated anti-human CD38 (BD Pharmingen, NJ, USA). Naive B-cells (CD19+, IgD+, CD38−), GC B-cells (CD19+, IgD−, CD38+) and memory B-cells (CD19+, IgD−, CD38+) were isolated using
a FACS aria (BD Biosciences, San Jose, USA). The cells were gated for single cells by forward and sideward scattering. After sorting, the B cell subsets were lysed in RNA-Bee (Tel-Test Inc. Frindswood, Texas, USA) and stored at -80ºC, until further processing.

**qRT-PCR and clustering analysis**

RNA was isolated from normal B-cell subsets with Nucleospin RNA 11 (Macherey-Nagel, Düren, Germany) as described previously (29). B-cell subsets RNA from 3 different tonsil samples were pooled together accordingly to rule out small differences related to genetic variation in individuals and differences related to differences in histology. We used a commercial available miRNA qRT-PCR profiling kit (Applied Biosystems, Foster City, USA), which at that time contained 183 miRNAs. We did not specifically select the miRNAs. qRT-PCR profiling for 183 mature miRNAs were carried out according to manufacturers’ protocols. Unsupervised clustering analysis was performed using Genesis (30). A heatmap was generated by mean normalization of experiments and genes, followed by average linkage clustering for all miRNAs that showed a cycle threshold (Ct) value of <35 in at least one of the three B-cell subsets. As the mean and median values for the three B-cell subsets were very similar (Ct value of 31.3-31.9), the mean of all miRNAs assessed was used for normalization (ΔCt = Ct_{miRNA} - Ct_{mean}). Relative expression levels were determined with the formula $2^{-\Delta Ct}$. Selection of GC B-cell specific miRNAs was performed by the following criteria: 1) miRNAs with $2^{-\Delta Ct}$ greater than 2 in at least one of the subsets, i.e. miRNAs with a relatively high expression level and 2) miRNA showing at least 4.5 fold difference among the subsets.

**miRNA in situ hybridization (ISH)**

MiRNA ISH was performed as reported previously (31). Briefly, 11 digoxigenin (DIG) labeled locked nucleic acid (LNA) probes antisense to miR-15b, miR-17-5p, miR-21, miR-25, miR-29a, miR-93, miR-106a, miR-146a, miR-122, miR-150 and miR-181b (Exiqon, Vedbaek, Denmark) were used for overnight hybridization on tissue
sections at 55°C. Detection was accomplished with anti-DIG alkaline phosphate Fab fragment followed by nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) color development (Roche, Switzerland). miR-122 was chosen as a negative control for staining in tonsil sections because it has been reported that the expression of miR-122 is detected only in liver (32).

**Immunohistochemistry (IHC)**

Slides were deparaffinized and endogenous peroxidase was blocked by incubation with 3% H$_2$O$_2$ for 10 minutes. Antigen retrieval was performed according to various protocols of the manufacturers. Immunostaining was performed using antibodies against Survivin (Cell Signaling Technology, Boston, MA), Foxp1 and c-Myb (Abcam, Cambridge, UK) at a dilution of 1:50-1:400. Signals were amplified by incubation with the appropriate Horseradish Peroxidase-conjugated antibodies for 60 minutes and the reactivity was visualized by diaminobenzidin.

**Transfection, Western blotting and DiOC6 staining**

The Epstein-Barr virus negative Burkitt lymphoma (BL) cell line DG75 (33) was cultured in RPMI 1640 supplemented with ultraglutamine, 100 U/ml penicillin/streptomycin, and 10% fetal calf serum (Cambrex Biosciences, Walkersville, USA). Transfection of DG75 with synthetic miR-150 mature duplex (Ambion, Austin, USA) was performed using the A-23 program of the Amaxa nucleofector I device (Amaxa, Gaithersburg, USA) with nucleofection solution V. 24h post transfection, half of the cells were lysed, separated in 10% SDS-polyacrylamide gel and immunoblotted for c-Myb, Foxp1 and Survivin with the same antibodies as used for IHC. 48h post transfection, the other half of the cells were incubated with 25 ug/mL DiOC6 at 37°C for 30 minutes, washed and analyzed by FACS. Cells showing high DiOC6 staining were gated as live cells while cells with low DiOC6 staining were regarded as apoptotic/death cells.
Results

miRNA profiling for normal B-cell subsets from tonsils

Out of 183 miRNAs assessed, 86 miRNAs were expressed (Ct<35) in at least one of the three normal B-cell subsets. A heatmap of these 86 miRNAs is given in Figure 1A. To identify miRNAs that are important throughout the germinal center response, we focused on the miRNAs that showed an at least 4.5 fold difference between GC and non GC B-cells, resulting in 16 miRNAs (Figure 1B). Eight of these 16 miRNAs, namely miR-15b, miR-93, miR-181b, miR-25, miR-17-5p, miR-106a, miR-28 and miR-130b showed increased expression levels in GC B-cells while miR-150, miR-29a, miR-320 and miR-223 showed a lower expression level in GC B-cells. Expression levels of miR-145 and miR-146 increased during the GC transit whereas the expression levels of miR-331 and let-7a decreased during GC transit (Figure 1B, Table 1).

Table 1. Germinal center specific miRNAs.

<table>
<thead>
<tr>
<th>Relative expression, $2^{-\Delta Ct}$</th>
<th>Fold change</th>
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<tbody>
<tr>
<td>naive B-cell</td>
<td>GC B-cell</td>
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<tr>
<td><strong>Highest in GC</strong></td>
<td></td>
</tr>
<tr>
<td>miR-15b</td>
<td>4.8</td>
</tr>
<tr>
<td>miR-93</td>
<td>2.2</td>
</tr>
<tr>
<td>miR-181b</td>
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<tr>
<td>miR-106a</td>
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<tr>
<td>miR-130b</td>
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</tr>
<tr>
<td><strong>Lowest in GC</strong></td>
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</tr>
<tr>
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<td>miR-223</td>
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<table>
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<tr>
<th>Change during GC transit</th>
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<tbody>
<tr>
<td>Increase</td>
</tr>
<tr>
<td>miR-145</td>
</tr>
<tr>
<td>miR-146</td>
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<tr>
<td>Decrease</td>
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<td>miR-331</td>
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<tr>
<td>let-7a</td>
</tr>
<tr>
<td>Consistent</td>
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<tr>
<td>miR-21</td>
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miRNA profile of B-cell subsets

Figure 1. miRNA profiling of naive, germinal center and memory B-cells. A) Heatmap of 86 miRNAs which were expressed (Ct<35) in at least one of the normal B cell subsets and showed differential expression among the subsets. Green color represents lower Ct value, ie. upregulation, while red color represents higher Ct value, ie. downregulation. B) 16 miRNAs showed at least 4.5 fold difference when compared to germinal center B-cells. The first 9 miRNAs and miR-21 (control with not more than 2 fold changes during the GC transit, not shown in graph) were selected for ISH.

miRNA in situ hybridization in tonsils

In view of the differential miRNA expression among the normal B-cell subsets and our main interest in GC B-cells, 9 miRNAs which were most abundant and differentially expressed in GC B-cells (the first 9 miRNA listed from the left, Figure 1B) were chosen for miRNA ISH. As differences in miR-21 expression level among the three B-cell subsets were minimal (~2 fold differences, Table 1), this miRNA was chosen as a control for ISH where we expect to see no variation in staining intensity.
within the GC structures. Meanwhile, the liver specific miR-122 probe was used as the negative control. Out of the 11 miRNAs chosen for ISH, staining for 4 miRNAs (miR-15b, miR-93, miR-25 and miR-29a) failed in all cases for unknown reasons. This failure was not related to the abundance of the miRNAs in GC B-cells as determined by qRT-PCR profiling.

As a negative control, ISH with miR-122, a liver specific probe showed negative staining in the tonsil tissue section (Figure 2). miR-21 showed no variation in staining intensities of GC structures and the mantle zone in tonsil tissue sections as expected. According to morphology and distribution pattern in the tonsil, cells with the strongest miR-21 staining were likely to be interfollicular T-cells (Figure 2). The proteinase K step in the ISH procedure precludes double staining of miR-21 and CD3, therefore we can only suggest that the cells with the strongest miR-21 staining in tonsils represent interfollicular T-cells based on their morphology and distribution pattern in the tonsil.

MiRNA ISH results showed weak cytoplasmic staining of miR-150 in GC as compared to the mantle zone, while miR-17-5p, miR-106a, and miR-181b stained stronger in GC structures (Figure 2). This indicates a higher expression of miR-17-5p, miR-106a and miR-181b and lower expression of miR-150 in GC B-cells as compared to naïve and memory B-cells located in the mantle zone (Figure 2). These
results were consistent with our miRNA profiling results. For the staining of miR-146a our miRNA ISH depicted a stronger staining of miR-146a in the germinal center (Figure 2), mainly contributed by a stronger staining in the centroblasts in the dark zone of the GC, as compared to a weaker signal in the mantle zone (Figure 3). Profiling showed a low expression in naive cells as compared to GC and memory B-cells, which showed an almost similar expression level. Since naive B-cells (low miR-146a levels) represent a major population in the mantle zone as compared to the memory cells (high miR-146a levels), this might explain the stronger staining observed in the GC B-cell dark zone (Figure 2).

Interestingly, variation in staining intensity was observed within the GC structures for several miRNAs. Gradual decrease of miR-17-5p, miR-106a, and miR-181b staining was observed from the dark to the light zone in the GC (Figure 2). miR-150 demonstrated the strongest staining in part of the centroblasts only in the dark zone, whereas the light zone, containing the centrocytes, stained even weaker than the naive and memory B-cells in the mantle zone (Figure 3).

**miR-150 expression levels and its possible role in B-cell homeostasis**

In view of the dynamic expression patterns of miR-150 throughout the germinal center reaction and its absence in proliferation centers of CLL cases (20), we investigated the possible role of miR-150 in regulation of B-cell homeostasis. One proven target for miR-150, c-Myb (14), and two putative miR-150 targets, Foxp1 (both PICTAR and TargetScan 4.2 predictions) and Survivin (not predicted by both
PICTAR and TargetScan 4.2 programs but contained miR-150 6mer seed complimentary site at its 3’ UTR), were examined by immunohistochemistry staining. The c-Myb and Survivin staining patterns were inversely correlated with miR-150 ISH staining patterns in tonsils (Figure 4A), supporting the targeting of these genes by miR-150. The staining pattern of Foxp1 did not show a clear inverse pattern with miR-150 ISH (Figure 4A). To confirm targeting by miR-150, the BL cell line DG75, which has a GC B-cell origin and low miR-150 expression level, was transfected with synthetic miR-150 mature duplex for induction of the miRNA (confirmed by qRT-PCR, data not shown). Western blot analysis of cell lysates showed repression of c-Myb, Survivin as well as Foxp1 in the cells transfected with miR-150 (Figure 4B). These results suggest that expression of miR-150 either directly or indirectly regulates the expression of c-Myb, Survivin and Foxp1, which are all genes important during B cell development (34;35). Also, DiOC6 analysis for DG75 cell lines 48h post transfection with miR-150 showed that there was an increase in the percentage of cells with low DiOC6 staining (Figure 4C). Although the increase was not statistically significant, the trend indicates that the amount of apoptotic/death cells increased upon transfection with synthetic miR-150 mature duplex.

Figure 4. miRNA target genes validation. A) Immunohistochemistry staining showing that the expression of c-Myb and Survivin but not Foxp1 were inversely correlated to miR-150 in tonsils. B) Downregulation of c-Myb, Survivin and Foxp1 upon miR-150 overexpression in DG75. C) Although not statistically significant, an increase in percentage of apoptotic/death cells (low DiOC6 staining) was observed when miR-150 was induced in DG75.
Discussion
Results of miRNA ISH on tonsil sections confirmed differential expression of several miRNAs as identified by the miRNA profiling work. More than 4.5 fold changes in expression levels of miR-17-5p, miR-106a, miR-146a, miR-150 and miR-181b were found in germinal center B-cells as compared to naïve and memory B-cells. A GC B-cell specific expression pattern was confirmed by miRNA ISH staining. To our knowledge, this is the first report confirming distinct miRNA expression patterns in tonsils with ISH. Recently, Malumbres et al. did a similar profiling to identify miRNA expression changes during the GC response, but only centroblasts and not centrocytes were used. 39 miRNAs are identified as the classifier to distinguish among T-cells, centroblasts, naive and memory B-cells (26). Our study examined the GC B-cells which included centroblasts and centrocytes, looked for miRNAs with the highest fold change (>4.5 fold) in naive or memory compared to GC B-cells (T-cells not included) and resulted in a total of 16 miRNAs. Despite the differences in cell sorting and profiling technique we observed a similar expression pattern for 28 out of their 39 classifier miRNAs (supplementary data 1). 12 of the 16 miRNAs showing the most extreme changes in expression levels during GC response in our study are included in their classifier miRNAs. We confirmed differences between GC and naive / memory B-cells for 5 miRNAs by ISH in tonsil tissue sections. We show that ISH is a powerful tool to study cell or compartment specific miRNA expression, which can easily be missed upon analysis from sorted cells.
MiRNA ISH revealed a distinct staining pattern in the GC structures with decreasing miR17-5p, miR-106a, and miR-181b levels from the dark zone to the light zone. This difference was most likely due to the differences in expression levels between centroblasts and centrocytes. CD77 is generally accepted as a marker to discriminate between centroblasts and centrocytes (36;37). In previously reported gene expression profiling studies of B-cells during GC transit, Klein et al. found thousands of differences among naive, germinal center and memory B-cells, but only 19 differentially expressed genes between CD77- and CD77+ GC B-cells (28). Consistent with these findings, Hogerkorp and Borrebaeck also found no differences in gene expression profiles between CD77- and CD77+ GC B-cells (27). Lack of
differences in these studies raised the question if CD77 can be used as a reliable marker to discriminate between centroblasts and centrocytes. In view of the dynamics of GC B-cells to shift from the dark to the light zone (38) and due to current limitations in isolating pure centroblasts and centrocytes, miRNA ISH in normal tissue represents the best tool to study putative differences in miRNA expression levels in these two closely related B-cell populations, as differences in ISH staining intensities were obviously seen in cells within the GC. MiR-17-5p and miR-106a are both members of the same seed family, namely the miR-17 family which also includes miR-17-5p, miR-20a, miR-20b, miR-93, miR-106a and miR-106b. These miRNAs share the same seed sequence and most likely target the same genes and hence exert similar effects. It has been shown that miRNAs from this miR-17 seed family negatively regulate the expression of cyclin dependent kinase inhibitor CDKN1A (also known as p21), allowing cells to overcome the G1 cell cycle checkpoint (39). As high expression levels of miR17-5p and miR-106a were observed specifically in centroblasts in the dark zone of GC whereas CDKN1A has been reported to be 30 fold down regulated in centroblasts, we suggest that these miRNAs are essential for centroblasts to progress from G1 to S phase of the cell cycle, by down regulating CDKN1A (27;28).

The expression level of miR-146a increases dramatically when naïve B-cells enter the germinal center and remains high when they exit from the germinal center. It has been reported that proinflammatory cytokines and microbial components can induce expression of miR-146a in the acute monocytic leukemia cell line, THP-1 (40). Luciferase reporter assays demonstrated that miR-146a is a nuclear factor-κB (NF-κB) dependent miRNA (40). By targeting IL-1 receptor associated kinase (IRAK1) and TNF receptor-associated factor 6 (TRAF6), miR-146a causes a negative feedback for the Toll-like receptor (TLR) signaling pathway (40). As the role of NF-κB in B-cell homeostasis has been implicated in both immature B cell and also in maintaining GC function (41), the strong miR-146a staining pattern we observed in GC correlates with the functional role of NF-κB in the GC. However, the exact function of miR-146a in this pathway and how it might affect B-cell survival should be further investigated.
miRNA profile of B-cell subsets

MiR-181b contains the same seed sequence with miR-181a. MiR-181a has been proven to play a role in hematopoietic differentiation, in favor of B-cells (13) and its expression is regulated during T-cell maturation (18). Recently, it has been shown that AID is a direct target of miR-181b and overexpression of miR-181b in primary splenic B cells reduces the class switch recombination rate (42). We observed dynamic expression of both miR-181a and miR-181b during the GC transit, where their expression levels peak during the GC stage and falls back to a level similar to that of naive B-cells upon exit from the GC. qRT-PCR profiling revealed a 2 fold and 5 fold enrichment in GC B-cells for miR-181a and miR-181b, respectively. These results indicate that both miR-181a and miR-181b from the same miRNA seed family are crucial for B-cell differentiation and fine tuning of the GC response.

Our group has previously demonstrated that miR-150 is highly expressed in the majority of CLL tumor cells, but not in the proliferation centers (20). CLL cells are considered to be of memory B-cell origin or derived from naive B-cells (43;44). The high miR-150 expression in CLL is consistent with the high miR-150 expression levels we found in naive and memory B-cells sorted from normal tonsil and the lack in proliferation centers is consistent with the low levels in GC structures of tonsil. Recently, a key role has been established for miR-150 in B-cell development (14;15). Overexpression of miR-150 blocks B-cell maturation by inhibiting the transition of cells from pro-B to pre-B stage and also induces a slight but significant increase in apoptotic rate (15). Consistent with this finding, Xiao et al. demonstrated that ectopic miR-150 expression in pro-B cells resulted in an increase in cell death (14). It was concluded that miR-150 exerts this effect by suppression of its target, c-Myb (14), a transcription factor which plays an important role during B-cell development, maintenance of proliferation as well as cell cycle control of hematopoietic cells (35;45-47). C-Myb has already been proven to be important for transition of pro-B to pre-B stage and also for maintenance of follicular B-cells, but interestingly, not for mantle zone B-cells (35). This is in line with our findings, where in the mantle zone, expression of c-Myb indeed was weak while expression of miR-150 that negatively regulates c-Myb was found to be relatively higher. Similarly, in tonsil sections, Survivin showed an inverse staining pattern with miR-150. For
Foxp1, however, such an inverse staining pattern was not obvious, because expression of Foxp1 was also expressed in the mantle zone. Nevertheless, it is still possible that Foxp1 is regulated by miR-150 since Foxp1 knockout mice shared a comparable pro-B to pre-B transition blockade phenotype with mice ectopically expressing miR-150 in hematopoietic cells (15;34). According to our Western blotting results, induction of miR-150 resulted in downregulation of c-Myb, Foxp1, and Survivin, supporting the targeting of these genes by miR-150. Nonetheless, we cannot exclude the possibility of an indirect regulation pathway for downregulation of Survivin and Foxp1.

It has been reported that c-Myc, which is implicated in the vast majority of Burkitt’s lymphoma, can induce the expression of the miR-17-92 cluster and repress the expression of miR-150 (48;49). In line with previous publications (14;15), we observed a trend of increased percentage of apoptotic/death cells upon induction of miR-150 in the Burkitt’s lymphoma derived cell line, DG75. Nevertheless, the increased percentage of apoptotic/death cells was not statistically significant. It can be speculated that the induction of miR-150 was not sufficient to suppress the c-Myc mediated lymphomagenesis as the expression levels of the oncogenic miR-17-92 cluster members remains high.

In conclusion, we have determined a miRNA profile of naive, GC and memory B-cells sorted from normal tonsils. MiRNAs that were differentially expressed have been identified and verified, for the first time, by miRNA ISH in tonsil tissue sections. Remarkably, miRNA ISH revealed variation in expression levels within the GC structures which indicates the involvement of differentially expressed miRNAs in the functional regulation of centroblasts and centrocytes. Our findings warrant future studies in centroblasts and centrocytes aiming at elucidating the exact pathways targeted by these and possibly other miRNAs.

Disclosure/Conflict of Interest/Acknowledgments

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