The roles of noncoding RNAs in B-cell lymphomas

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Chapter 3

MiR-378a-3p is critical for Burkitt lymphoma cell growth

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Cancers, in revision
Abstract

MicroRNAs (miRNAs) are small RNA molecules with important gene regulatory roles in normal and pathophysiological cellular processes. Burkitt lymphoma (BL) is a MYC-driven lymphoma of germinal center B (GC-B) cell origin. To gain further knowledge on the role of miRNAs in the pathogenesis of BL, we performed small RNA sequencing in BL cell lines and normal GC-B cells. This revealed 26 miRNAs with significantly different expression levels. For five miRNAs, the differential expression pattern was confirmed in BL cell lines and primary tissues compared to GC-B cells. MiR-378a-3p was upregulated in BL and its inhibition reduced the growth of multiple BL cell lines. RNA immunoprecipitation of Argonaute 2 followed by microarray analysis (Ago2-RIP-Chip) upon inhibition and ectopic overexpression of miR-378a-3p revealed 63 and 20 putative miR-378a-3p targets, respectively. Effective targeting by miR-378a-3p was confirmed by luciferase reporter assays for MNT, FOXP1, IRAK4, and IncRNA JPX, all of which have been implicated in proliferation and cancer. In summary, we identified miR-378a-3p as a miRNA with an oncogenic role in BL and identified four novel miR-378a-3p target genes, i.e. MNT, FOXP1, IRAK4, and JPX.

Keywords: Burkitt lymphoma; miR-378a-3p; cell growth; microRNA

Introduction

Burkitt lymphoma (BL) is one of the fastest growing human tumors with a cell doubling time of about 24 hours. BL mainly affects children and young adults but can also occur at later age [1]. The tumor cells are derived from germinal center B (GC-B) cells and usually carry the hallmark translocation involving MYC and the immunoglobulin heavy or light chain loci which results in high expression of MYC [2, 3].

MicroRNAs (miRNAs) are a class of short non-coding RNAs of about 22 nucleotides. They modulate gene expression at the post-transcriptional level by translational inhibition or by inducing mRNA degradation [4, 5]. MiRNAs regulate a wide range of cellular processes, including cell cycle, proliferation, and apoptosis and are important determinants of B-cell development and maturation [6]. A widespread deregulation of miRNA expression has been observed in all B-cell lymphoma subtypes [7].

We and others have identified distinct miRNA expression patterns in BL and demonstrated the central role of MYC in regulating miRNA levels [8-12]. Functional studies showed crucial roles for the miR-17~92 cluster, miR-28, miR-150, and miR-155 as either oncogenic or tumor suppressor miRNAs in the pathogenesis of BL [9, 13-19]. Still, the role of most of the deregulated miRNAs in BL remains to be explored.
MYC-induced miR-378a-3p is essential for BL growth

In this study we carried out small RNA-sequencing in BL cell lines and normal GC-B cells and subsequently focused on downstream functional experiments for miR-378a-3p. We show for the first time that this miRNA is upregulated in BL and confirmed its regulation by MYC. Further analysis indicated that miR-378a-3p is essential for growth of BL cells. Genome wide target gene identification revealed four novel targets of miR-378a-3p, including MNT, FOXP1, IRAK4 and JPX, with known functions related to cell growth.

Results

MiRNA expression profiling in BL and GC-B cells

An overview of the total number of reads and percentages of mapped reads per sample is given in Table S1. The top-10 most abundantly expressed miRNAs accounted for 73% of all reads in BL and for 71% in GC-B cells (total GC-B cells sorted based on a +IgD-CD38+ or IgD-CD138-CD3-CD10+ phenotype). Seven of the top-10 most abundantly expressed miRNAs were shared between BL and GC-B cells (Figure 1A). Twenty-six miRNAs were significantly differentially expressed between BL and GC-B cells, including eight miRNAs upregulated in BL and 18 downregulated (Figure 1B). qRT-PCR validation on the same set of samples confirmed differential expression for six out of eight selected miRNAs (Figure 1C and Figure S1). Of the six validated miRNAs, miR-378a-3p levels were increased in BL relative to GC-B cells, while expression levels of miR-28-5p, miR-155-5p, miR-363-3p, miR-221-3p, and miR-222-3p were decreased. Further expression analysis in primary BL tissue samples and GC-B cells confirmed the differential expression for five of the six miRNAs, excluding miR-221-3p (Figure 1D).

MYC-induced miR-378a-3p controls BL cell growth

We selected miR-378a-3p for further functional analysis, because it was the only significantly upregulated miRNA with a high expression level in BL. Previous studies demonstrated that miR-378a-3p is induced by MYC in human mammary epithelial cells [20]. We assessed the regulatory role of MYC in B cells using the P493-6 B-cell model that has a tetracycline-repressible MYC allele [21]. Our results showed that this miRNA is also induced by MYC in B cells (Figure 2A).

To explore the role of miR-378a-3p in growth of BL cells, we inhibited miR-378a-3p using a lentiviral miRNA inhibition construct (mZip-378a-3p) in 4 BL cell lines and followed cell growth in a GFP competition assay. Compared to the negative control (mZip-SCR), a significant decrease in the number of GFP-positive cells was observed in three (ST486, CA46, and DG75) out of four BL cell lines (Figure 2B). No relation was observed between the reduction in the percentage of GFP+ cells and the level of
miR-378a-3p expression (Figure S2). Together, our data indicate that inhibition of miR-378a-3p is disadvantageous for BL cells, suggesting miR-378a-3p is indispensable for growth of BL cells.

Overexpression of miR-378a in ST486 using a lentiviral miRNA overexpression construct (pCDH-378a) resulted in a ~47-fold increase in miR-378a-3p level. In a GFP competition assay miR-378a overexpression had no effects on cell growth, probably due to the already high endogenous levels (data not shown).

Figure 1. Deregulated expression patterns of miRNAs in BL compared to GC-B cells. (A) Overview of the top-10 most abundantly expressed miRNAs in Burkitt lymphoma (BL) and normal germinal center B (GC-B) cells as determined by small RNA-sequencing. Asterisks indicate miRNAs present in both the top-10 of BL and GC-B cells. (B) Heatmap of miRNAs significantly differentially expressed between BL and GC-B cells. (C) qRT-PCR validation results for 6 of the 8 tested miRNAs with significantly differential expression between BL cell lines and GC-B cells. MiRNA expression levels
were normalized to RNU44. (D) The differential expression pattern was confirmed for 5 of the 6 tested miRNAs when BL tissues and GC-B cells were compared. MiRNA expression levels were normalized to RNU49. Significant differences were calculated using unpaired T-test. *P < 0.05, **P < 0.01, and ***P < 0.001.

**Figure 2. MYC-induced miR-378a-3p is essential for BL cell growth.** (A) Levels of MYC and miR-378a-3p in tetracycline treated (“-”, MYC-off) and non-treated (“+”, MYC-on) P493-6 B-cells. MYC levels were normalized to RPII. MiRNA levels were normalized to RNU44. (B) Green fluorescent protein (GFP) growth competition assay upon miR-378a-3p inhibition in 4 Burkitt lymphoma (BL) cell lines. The miR-378a inhibitor (mZip-378a-3p) and the scrambled control (mZip-SCR) were stably transduced in BL cells using a lentiviral vector, which co-expresses GFP. The GFP percentage was measured for 22 days, and the GFP percentage at the first day of measurement (day 4) was set to 100%. All assays were performed in triplicate. Significant differences were calculated using a mixed model analysis. ***P < 0.001 and ****P < 0.0001.

**Identification of miR-378a-3p targets**

To identify miR-378a-3p target genes, we performed Ago2-RIP-Chip upon miR-378a-3p inhibition and overexpression in ST486 cells (Figure S3A and B). Efficient pulldown of Ago2-containing RISC and miRNAs was confirmed by qRT-PCR for miR-378a-3p and the unrelated highly expressed miR-181a-5p (Figure S3C and D) and by western blot for Ago2 protein (Figure S3E and S4). The number of Ago2-IP enriched probes was similar in all 4 conditions, ranging between 6.3%-9.8% of the consistently expressed probes (Table 1).

A total of 22 probes corresponding to 20 genes showed a ≥ 2-fold increased IP-enrichment upon miR-378a overexpression compared to empty vector control infected cells (Figure 3A and Table 2). Nine of the 20 genes (45%) had at least one putative miR-378a-3p binding site (7mer-A1, 7mer-m8, or/and 8mer). Six of them i.e. MNT, HSPB1, IRAK4, CCNK, CDKN2A, and RNF34, had a gene ontology (GO) term related to cell growth, apoptosis, and/or cell cycle. Upon miR-378a-3p inhibition, 74
probes, corresponding to 63 genes showed a ≥ 2-fold decreased IP-enrichment compared to the negative control infected cells (Figure 3B and Table 3). Nineteen of these 63 genes (30%) contained at least one putative miR-378a-3p binding site, including CISH, BCR, TUBA1C, SMARCA4, and FOXP1 with a GO term related to cell growth, apoptosis, or cell cycle. One of the target genes, i.e. MYCBP, was identified with both experimental setups.

Table 1. Number of genes in the miRNA targetome upon miR-378a-3p overexpression (pCDH) and inhibition (mZip)

| IP/T ratio | pCDH (n=9,233) | mZip (n=8,944) |
|------------|----------------|----------------|
|            | EV 378a | 378a/EV | SCR mZip | 378a-3p | SCR/mZip | 378a-3p |
| ≥ 2        | 611     | 586    | 20       | 741     | 878     | 63       |
| ≥ 4        | 117     | 117    | 2        | 171     | 196     | 4        |
| ≥ 8        | 25      | 18     | 0        | 53      | 34      | 0        |

EV = pCDH-EV, 378a = pCDH-378a, SCR = mZip-SCR.

Table 2. Identified targets of miR-378a-3p upon overexpression

| Gene   | Transcript ID | IP/T ratio EV* | miR-378a-3p binding site | Growth-related GO |
|--------|---------------|----------------|--------------------------|-------------------|
|        |               | 378a | FC | 5’UTR | CDS | 3’UTR |       |
| IRAK4  | ENST00000613694 | 1.0  | 4.6 | 4.6 | 8m  |       | yes   |
| CDKN2A | ENST00000304494 | 1.0  | 4.0 | 4.0 |     |       | yes   |
| JPX    | ENST00000415215 | 1.5  | 5.8 | 3.9 | 8m* |       |       |
| PLGRTK | ENST00000223864 | 1.0  | 3.0 | 3.0 | 8m  |       |       |
| TMEM245| ENST00000374586 | 1.0  | 2.9 | 2.9 | 7m8/8m |       |       |
| TOMM6  | ENST00000398884 | 1.5  | 4.1 | 2.7 |       |       |       |
| CDK1   | ENST00000395284 | 1.0  | 2.6 | 2.6 |       |       |       |
| FAM117A| ENST00000240364 | 1.4  | 3.6 | 2.6 |       |       | yes   |
| WDR83OS| ENST00000596731 | 1.3  | 3.2 | 2.5 | 7m8  |       |       |
| CCNK   | ENST00000389879 | 1.0  | 2.4 | 2.4 | 8m   |       | yes   |
| MYCBP  | ENST00000397572 | 2.3  | 5.4 | 2.3 | 7mA1 |       |       |
| RNF34  | ENST00000392465 | 1.3  | 3.0 | 2.3 |       |       | yes   |
| UBC    | ENST00000396467 | 1.0  | 2.3 | 2.3 |       |       | yes   |
| POP4   | ENST00000585603 | 1.0  | 2.3 | 2.3 |       |       |       |
| MNT    | ENST00000174618 | 1.2  | 2.7 | 2.3 | 7m8  | 7mA1  | yes   |
| HSPB1  | ENST00000248553 | 1.8  | 3.9 | 2.2 | 7mA1 |       | yes   |
| INAFM1 | ENST00000552360 | 1.3  | 2.8 | 2.2 |       |       |       |
| PCNA   | ENST00000379160 | 1.0  | 2.1 | 2.1 |       |       | yes   |
| SP100  | ENST00000264052 | 1.2  | 2.5 | 2.1 |       |       |       |
| RPP25L | ENST00000297613 | 1.2  | 2.4 | 2.0 |       |       |       |

*IP/T ratios in pCDH-EV (EV) were set to 1.0 in case the ratios were < 1.0.
**The binding site in the noncoding RNA has been listed in the 3’-UTR column.
7mA1 = 7mer-A1, 7m8 = 7mer-m8, 8m = 8mer, FC = fold change, and 378a = pCDH-378a, IP = Ago2-immunoprecipitation fraction, T = Total fraction.
### Table 3. Identified targets of miR-378a-3p upon inhibition

| Gene       | Transcript ID     | IP/T ratio | miR-378a-3p binding site | Growth-related GO | 5'UTR | CDS | 3'UTR |
|------------|-------------------|------------|--------------------------|------------------|-------|-----|-------|
|            |                   | mZip       | SCR                      |                  |       |     |       |
| DYNLRB1    | ENST00000357156   | 1.0        | 6.4                      | 6.4              |       |     |       |
| VPS18      | ENST00000220509   | 8.5        | 43.4                     | 5.1              |       |     |       |
| NAPA-AS1   | ENST00000594367   | 1.2        | 5.2                      | 4.3              | 7mA1**|     |       |
| C11orf95   | ENST00000432688   | 1.0        | 4.2                      | 4.2              |       |     |       |
| HOMEZ      | ENST00000357460   | 1.2        | 4.3                      | 3.5              |       |     |       |
| TMEM79     | ENST00000405535   | 1.7        | 5.7                      | 3.4              |       |     |       |
| FOXP1      | ENST00000493089   | 4.5        | 14.9                     | 3.3              | 7mA8m| yes |       |
| PCIF1      | ENST00000372409   | 1.0        | 3.2                      | 3.2              | 7mA8| yes |       |
| ATP6VOC    | ENST00000330398   | 1.0        | 3.1                      | 3.1              |       |     |       |
| CISH       | ENST00000348721   | 2.1        | 6.4                      | 3.1              | 8mA1| yes |       |
| Inc-FOXB1-8| ENST00000334346   | 1.0        | 3.3                      | 3.0              | 7mA1| yes |       |
| MTIB       | ENST00000525151   | 1.0        | 2.7                      | 2.7              |       |     |       |
| Inc-EGLN1-1| ENST00000382882   | 1.0        | 3.0                      | 2.9              |       |     |       |
| NELFA      | ENST00000565768   | 1.2        | 3.2                      | 2.8              |       |     |       |
| BCR        | ENST00000409404   | 1.0        | 2.7                      | 2.7              | 8mA1| yes |       |
| MT1L       | ENST00000409404   | 1.0        | 2.7                      | 2.7              |       |     |       |
| FAT3       | ENST00000409404   | 1.0        | 2.7                      | 2.7              |       |     |       |
| TRAF3P2-AS1| ENST00000525151   | 1.0        | 2.7                      | 2.7              |       |     |       |
| C22orf39   | ENST00000611555   | 1.0        | 2.6                      | 2.6              |       |     |       |
| KRTCAP2    | ENST00000295682   | 1.6        | 4.1                      | 2.6              |       |     |       |
| LINC01122  | ENST00000427421   | 11.2       | 29.4                     | 2.6              | 7mA1**| yes |       |
| ACTG1F20   | ENST00000214547   | 1.3        | 3.2                      | 2.5              |       |     |       |
| Inc-KRTAP5-10-1| ENST0000030601 | 1.1        | 2.8                      | 2.4              |       |     |       |
| MT1E       | ENST00000397061   | 1.0        | 2.5                      | 2.4              | 7mA1| yes |       |
| NUDT19     | ENST00000391941   | 1.0        | 2.4                      | 2.4              |       |     |       |
| PRDX4      | ENST00000379341   | 1.0        | 2.4                      | 2.4              |       |     |       |
| TMEM258    | ENST00000537328   | 1.1        | 2.6                      | 2.4              |       |     |       |
| XLOC_12_005952| TCONS_12_00011050| 6.6        | 15.6                     | 2.4              | 7mA8| yes |       |
| BTG3       | ENST00000629582   | 1.6        | 3.7                      | 2.3              |       |     |       |
| EVISL      | ENST00000270530   | 1.3        | 3.0                      | 2.3              |       |     |       |
| LINC01534  | ENST00000432322   | 1.0        | 2.3                      | 2.3              |       |     |       |
| Inc-ADA-1  | ENST0000030601    | 1.1        | 2.8                      | 2.4              |       |     |       |
| Inc-ZNF431-4| TCONS_00019776   | 1.5        | 3.5                      | 2.4              |       |     |       |
| MT1A       | ENST00000290705   | 2.5        | 5.6                      | 2.3              |       |     |       |
| CSRP2      | ENST00000311083   | 2.7        | 5.9                      | 2.2              |       |     |       |
| FUCO1      | ENST0000026137    | 1.0        | 2.2                      | 2.2              | 7mA8| yes |       |
| HYAL3      | ENST00000336307   | 2.0        | 4.3                      | 2.2              |       |     |       |
| KCNQ1      | ENST00000632153   | 1.0        | 2.2                      | 2.2              |       |     |       |
| Inc-RP11-15894.1-2| TCONS_0019776| 1.5        | 3.3                      | 2.2              |       |     |       |
| PCNX       | ENST00000304743   | 3.8        | 8.3                      | 2.2              | 7mA1/8mA| yes |       |
| PRSS36     | ENST00000268281   | 1.4        | 2.8                      | 2.2              |       |     |       |
| SMARCA4    | ENST0000034626    | 1.0        | 2.2                      | 2.2              | 7mA8| yes |       |
| TNRC5C     | ENST00000335749   | 8.5        | 18.3                     | 2.2              | 7mA8| yes |       |
| TOLLIP     | ENST00000317204   | 1.3        | 2.8                      | 2.2              |       |     |       |
| ARF4       | ENST00000303436   | 1.0        | 2.1                      | 2.1              |       |     |       |
| ATG4D      | ENST00000309469   | 1.4        | 2.8                      | 2.1              |       |     |       |
| CSE1L      | ENST00000262982   | 1.2        | 2.5                      | 2.1              |       |     |       |
| Inc-PCF11-1| ENST00000262027   | 1.7        | 3.6                      | 2.1              |       |     |       |

**MYC-induced miR-378a-3p is essential for BL growth**

**3p is essential for BL growth**

**7mA1**

**7mA1/8mA**

**7mA8**

**7mA8m**

**GO**

**FC**
### Validation of miR-378a-3p targets by luciferase reporter assay

For further analysis, we selected MYCBP that was identified in both approaches and 6 candidates that had at least one 7mer-A1, 7mer-m8, or an 8mer and a GO term related to cell growth, apoptosis, or/and cell cycle (CISH, BCR, TUBA1C, FOXP1, MNT, and IRAK4). We also included the lncRNA JPX, as it showed a strong enrichment upon miR-378a-3p overexpression and contained an 8-mer seed binding site (Figure 3C).

To confirm targeting of the 8 selected genes by miR-378a-3p, we carried out luciferase reporter assays for the 10 putative miR-378a-3p binding sites in ST486 and DG75. This revealed a strong reduction in the Renilla/Firefly ratio for four of the miR-378a-3p binding sites in four genes (IRAK4, FOXP1 (site 1), MNT, and JPX) (Figure S5). To further confirm specific binding by miR-378a-3p we generated constructs with mutations in these four miR-378a-3p binding sites. For IRAK4 (trend), FOXP1 and MNT (both significant) wildtype binding sites showed lower Renilla/Firefly ratios compared to the mutated binding sites (black bars in Figure 3D), indicating binding of endogenous miR-378a-3p to these sequences. Upon miR-378a-3p overexpression a significantly reduced Renilla/Firefly ratio was observed for the wildtype, but not to the mutated target sites confirming efficient and specific targeting (Figure 3D). Taking together, these results confirmed targeting of IRAK4, FOXP1, MNT, and the lncRNA JPX by miR-378a-3p.

| Gene     | Transcript ID  | IP/T ratio | miR-378a-3p binding site | Growth-related GO |
|----------|----------------|------------|--------------------------|------------------|
| NANS     | ENST00000210444| 1.0        | SCR 1.0                  | 5'UTR            |
| ORM DL2  | ENST00000243045| 1.1        | SCR 2.1                  | 5'UTR            |
| PFKFB2   | ENST00000367080| 1.4        | SCR 3.0                  | 5'UTR            |
| PGM2L1   | ENST00000298198| 1.8        | SCR 3.7                  | 5'UTR            |
| PTPN23   | ENST00000265562| 1.5        | SCR 3.1                  | 5'UTR            |
| TSACC    | ENST00000368255| 1.5        | SCR 3.1                  | 5'UTR            |
| TUBA1C   | ENST00000301072| 1.1        | SCR 2.4                  | 5'UTR            |
| XLOC_l2_013031 | TCONS_l2_00024809 | 6.5 | SCR 13.7                 | 5'UTR            |
| C1orf61  | ENST00000342683| 1.3        | SCR 2.6                  | 5'UTR            |
| LAT      | ENST00000360872| 1.0        | SCR 2.0                  | 5'UTR            |
| LOC101929494 | N/A              | 1.6        | SCR 3.2                  | 5'UTR            |
| MYCBP    | ENST00000397572| 1.5        | SCR 3.1                  | 5'UTR            |
| NDRG4    | ENST00000394279| 1.1        | SCR 2.2                  | 5'UTR            |
| TUBE1    | ENST00000386662| 1.1        | SCR 2.2                  | 5'UTR            |

*IP/T ratios in mZip-378a-3p were set to 1.0 if < 1.0.

**Binding sites on noncoding RNAs have been listed in 3'-UTR column.

7mA1 = 7mer-A1, 7m8 = 7mer-m8, 8m = 8mer, FC = fold change, SCR = mZip-SCR, and N/A = not available, IP = Ago2-immunoprecipitation fraction, T = Total fraction.
Figure 3. Identification and validation of miR-378a-3p targets. MiR-378a-3p targets identified by Ago2-RIP-Chip upon (A) miR-378a overexpression relative to empty vector (EV) and (B) scrambled vector relative to miR-378a-3p inhibition (SCR/mZip-378a). Black bars in panels A and B indicate genes with miR-378a-3p seed binding sites. (C) Schematic representations of the 8 genes selected for luciferase reporter assay validation. Black boxes indicate positions of the open reading frames (ORF). Positions and types of miR-378a-3p binding sites are indicated relative to the ORF. * this binding site was not tested. (D) Luciferase reporter assay results upon co-transfection of ST486 and DG75 cells with the Psi-check-2 construct containing the wildtype (WT) or mutated (MUT) miR-378a-3p binding sites from the selected genes and either miR-378a-3p mimic or a negative control mimic. Significant differences were calculated using a paired t-test. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.
Discussion

In this study, we identified 26 miRNAs differentially expressed in BL cell lines compared to GC-B cells. For five of the miRNAs, deregulated expression levels were confirmed in both BL cell lines and primary tissues. Among them, miR-378a-3p is MYC-induced, highly abundant (top-10 within BL) and overexpressed in BL compared to GC-B cells. Inhibition of miR-378a-3p showed a negative effect on BL cell growth. In a genome-wide Ago2-RIP-Chip analysis, 20 and 63 genes were identified as the potential targets of miR-378a-3p upon miR-378a-3p overexpression and inhibition, respectively. Four genes i.e. MNT, FOXP1, IRAK4, and the lncRNA JPX were confirmed as novel targets of miR-378a-3p in BL.

Six of the 26 identified differentially expressed miRNAs were reported to be deregulated in BL by Oduor et al. in a previous study [8]. These six included miR-155-5p, miR-221-3p, miR-222-3p, and miR-28-5p which we have validated by qRT-PCR in BL cell lines and tissue samples. Nine additional miRNAs were proven to be differentially expressed in BL compared to other B-cell lymphomas [9-12, 20]. Thus, our small RNA sequencing data confirmed some of the previously identified deregulated miRNAs in BL. Moreover, we showed for the first time that miR-378a-3p is a MYC-induced and significantly upregulated miRNA in BL. Since the role of miR-378a-3p in BL was not studied before, we focused on this miRNA for further functional analysis.

Inhibition of miR-378a-3p resulted in a strong reduction of BL cell growth, suggesting a possible oncogenic role of miR-378a-3p in BL. The effect on growth upon miR-378a-3p was most pronounced in ST486. DG75 and CA46 showed intermediate phenotypes while no significant effect was observed in Ramos. There was no obvious relation between endogenous miR-378a-3p levels and the decrease in the percentage of GFP+ cells upon miR-378a-3p inhibition. Given the complex interactions between miRNAs and target genes, i.e. multiple targets per miRNA and multiple miRNAs per target, the differences in the observed phenotypes might be related to endogenous levels of either other miRNAs or target genes. Previous studies have shown opposite roles of miR-378a-3p in different cancer types. MiR-378a-3p was reported to inhibit growth or promote apoptosis and thus act as a tumor suppressor in colorectal cancer, lung cancer, ovarian cancer, prostate cancer and rhabdomyosarcoma [22-26]. In contrast, and in line with our findings, miR-378a-3p was shown to promote proliferation and reduce apoptosis in gastric cancer, nasopharyngeal carcinoma, colorectal cancer, and acute myeloid leukemia [27-30].

Using an unbiased genome-wide experimental approach we identified 83 putative miR-378a-3p target genes. Luciferase reporter assays confirmed targeting of 4 out of
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8 selected genes i.e. protein coding genes MNT, IRAK4, FOXP1, and the IncRNA JPX.

Previous studies showed a dual role of MNT in tumorigenesis. On the one hand, MNT was reported as a facilitator of MYC-driven T-cell proliferation and survival [31]. In line with this, a study in Eμ-MYC mice showed that reduced MNT levels reduced tumorigenesis [32], suggesting that MNT is indispensable for MYC-driven oncogenesis. However, in most studies MNT acted as a tumor suppressor and was a functional antagonist of MYC by repressing its activities related to cell cycle, proliferation, and apoptosis [33, 34]. Loss of MNT in mouse embryonic fibroblasts (MEFs) phenocopied the effect of MYC overexpression [35-37]. These findings are in line with our results and suggest that high levels of miR-378a-3p could promote BL tumorigenesis by reducing MNT levels and thereby enabling MYC to execute its oncogenic effects in BL.

IL-1 receptor–associated kinase 4 (IRAK4) plays an essential role in the Toll-like receptor (TLR) pathway [38,39], which mediates inflammatory signals in B cells and causes activation of NF-κB. The TLR pathway is hyperactive in mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL), and activation of NF-κB promotes B-cell survival and proliferation [40-42]. Depletion of IRAK4 showed a negative effect on NF-κB activity and autocrine IL-6/IL-10 engagement of the JAK-STAT3 pathway, reducing survival of DLBCL cells [43,44]. Despite the pro-survival role of NF-κB in DLBCL, activation of NF-κB has been reported to be disadvantageous in MYC positive BL consistent with our data and supporting a role of miR-378a-3p-dependent repression of IRAK4 to limit activation of NF-κB [45,46].

FOXP1 as a member of the forkhead box (Fox) transcription factor family plays critical roles in organ development, cell division, survival, and metabolism in multiple tissues and in particular in the immune system [47,48]. It is a regulator of early B-cell development and is associated with expression of B-lineage genes [49]. FOXP1 overexpression has been correlated with poor prognosis in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) [50-53], and reported to promote cell proliferation, cell cycle, and/or inhibit apoptosis of cervical cancer, myeloma, and glioma cells [54-56], indicating a pro-tumorigenic role. In contrast and more in line with our work, FOXP1 was described to have tumor suppressor like functions in lung and bladder cancer [57]. Interestingly FOXP1 expression is downregulated during the normal GC reaction and FOXP1 levels in BL are lower than in other B-cell lymphomas, comparable to GC-B cells [58,59]. Thus, the overexpression of miR-378a-3p may contribute to maintain low FOXP1 levels in BL. The potential relevance of low FOXP1 is further supported by the finding that aberrant expression of FOXP1 cooperates with (constitutive) NF-κB activity [60] which might be disadvantageous for the survival of BL.
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The long non-coding RNA JPX is an activator of XIST and acts as a molecular switch for X chromosome inactivation [61]. JPX was reported to act as an oncogene in ovarian cancer and non-small-cell lung cancer by promoting cell proliferation, invasion, and migration [62,63]. In hepatocellular carcinoma, JPX-dependent induction of XIST suppresses hepatocellular carcinoma progression by binding to the miR-155-5p oncomiR [64]. Current studies on JPX are limited and its potential role in BL remains unclear.

In summary, we identified 26 differentially expressed miRNAs. We confirmed the differential and high expression of miR-378a-3p in BL and showed a significant negative effect of miR-378a inhibition on BL growth. The growth-promoting role might be related to regulation of one or multiple of the identified miR-378a-3p targets MNT, IRAK4, FOXP1, and JPX.

Conclusions

In conclusion, we identified 26 miRNAs differentially expressed between BL cells and GC-B cells and confirmed deregulated expression of 5 out of 8 miRNAs both in BL cell lines and tissue samples. For one of the differentially and highly abundant (top-10 in BL) miRNAs, miR-378a-3p, we showed a negative effect on BL cell growth upon inhibition and identified 4 novel target genes. By targeting these genes, i.e. IRAK4, MNT, FOXP1, and JPX, miR-378a may control BL cell growth.

Materials and Methods

BL cell lines, germinal center (GC) B cells, and BL patient material

BL cell lines were purchased from ATCC (ST486 and Ramos) and DSMZ (CA46 and DG75). BL cells were cultured at 37°C under an atmosphere containing 5% CO2 in RPMI-1640 medium (Cambrex Biosciences, Walkersville, MD, USA) supplemented with 2mM ultra glutamine, 100U/ml penicillin, 0.1mg/ml streptomycin and 10% (CA46, DG75, and Ramos) or 20% (ST486) Fetal Bovine Serum (Sigma-Aldrich, Zwijndrecht, The Netherlands). P493-6 B-cells were cultured as described previously [65]. We routinely confirmed cell line identity using the PowerPlex® 16HS System (Promega, Leiden, The Netherlands) and absence of mycoplasma contamination.

GC-B cells and frozen BL tissue sections were obtained previously as described in [9,65,66]. GC-B cells (defined as CD20+IgD-CD38+, n=6 or IgD-CD138-CD3-CD10+, n=1) were sorted from routinely removed tonsil specimens of children. Specifically, for small RNA seq experiments FACS sorted (CD20+IgD-CD38+, n=2) and MACS sorted (IgD-CD138-CD3-CD10+, n=1) GC-B cells were used as controls, while FACS sorted (CD20+IgD-CD38+, n=4) GC-B cells were used for qRT-PCR validation experiments.
Written permissions for the use of the tonsil tissues to isolate GC-B cells were obtained from the parents of the children. The procedures followed for the BL tissue samples were according to the guidelines of the medical ethics board of the University Medical Center Groningen.

**RNA isolation**

RNA was isolated using miRNeasy Mini or Micro kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. RNA concentration was measured by a NanoDropTM 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA) and integrity was evaluated on a 1% agarose gel.

**Small RNA library preparation and sequencing**

1-2 µg total RNA from 4 BL cell lines and 3 samples of GC-B cells was used to generate small RNA libraries using Truseq Small RNA Sample Preparation Kit and TruSeq small RNA indices (Illumina, San Diego, CA, USA). Sequencing was performed on an Illumina 2000 HiSeq platform. After removal of 3’- and 5’-adaptor sequences from the raw reads using the CLC Genomics Workbench (CLC bio, Cambridge, MA, USA), sequencing data were analyzed using miRDeep version 2.0 (Max Delbrück Center for Molecular Medicine in the Helmholtz Association, https://www.mdc-berlin.de/8551903/en) [67] and annotated against miRbase version 21 (http://www.mirbase.org) [68] allowing one nucleotide mismatch. Read counts of miRNAs with the same mature miRNA sequence were merged. Total read counts per sample were normalized to 1,000,000. For statistical analysis, we included all unique miRNAs with at least 50 read counts in all seven samples. Genesis software v1.7.6 (Institute for Genomics and Bioinformatic Graz, Graz, Austria) was used to generate the heat map. The small RNA sequencing data were deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo; accession number GSE92616).

**qRT-PCR**

For validation of small RNA sequencing results, we selected differentially expressed miRNAs with expression log2 RPM > 8 in BL or GC-B cells. We selected the most optimal Taqman assay based on isoform abundance as observed in the small RNA sequencing data (Table S2). The miRNA expression levels were analyzed using Taqman miRNA quantitative PCR assays (Thermo Fisher Scientific Inc.) in a multiplexed fashion as described previously [69]. Cycle crossing point (Cp) values were determined with Light Cycler 480 software version 1.5.0 (Roche, Basel, Switzerland). Relative expression levels of miRNAs to house-keeping gene (RNU44
or RNU49) were determined by calculating $2^{-\Delta \Delta Cp}$ ($\Delta \Delta Cp = Cp_{miRNA} - Cp_{house-keeping\ gene}$). MYC transcript levels were analyzed as indicated previously [65].

**Lentiviral constructs, transduction and GFP competition assay**

Lentiviral constructs to inhibit (mZip-378a-3p) or overexpress (pCDH-miR-378a) miR-378a-3p were purchased from System Biosciences (Palo Alto, CA, USA). A non-targeting mZip-scrambled (SCR) and an empty vector pCDH (EV) construct were used as negative controls. Lentiviral particles were produced in HEK-293T cells by calcium phosphate precipitation transfection using a third-generation packaging system as described previously [66]. Briefly, HEK-293T cells were seeded in 6-well plates and grown till ~80% confluence. A plasmid mix consisting of 15μl CaCl2 (2.5M), 1μg pMSCV-VSV-G, 1μg pRSV.REV, 1μg pMDL-gPRRE, 2μg lentiviral vector, and 150μl of 2xHBS was prepared to transfect the HEK-293T cells. Virus was harvested and filtered by a 0.45μm filter 48 hours after transfection. Virus was either used directly or stored at -80°C.

For GFP competition assays, BL cell lines were infected with the mZip-378a-3p and the negative control mZip-SCR in three biological replicates per construct, aiming at an infection efficiency of 20% to 50% GFP+ cells on day 4. The percentage of GFP+ cells was monitored by flow cytometry (BD Biosciences, San Jose, CA, USA) three times per week for a total period of 22 days.

**Ago2-IP procedure**

Immunoprecipitation (IP) of the Ago2-containing RISC (Ago2-IP) procedure was done as described previously [70]. To identify miR-378a-3p target genes we applied Ago2-RIP-Chip on BL cells infected with lentiviral miR-378a-3p inhibition or overexpression constructs. For both constructs a parallel infection with appropriate control constructs (non-targeting or empty vector) was performed. We aimed at a high infection percentage and harvested the cells at day 5, either directly or after sorting to reach a GFP+ percentage > 95% for inhibition and > 85% for overexpression. For each AGO2-IP experiment we started with ~30 million cells. RNA was isolated from the Ago2-IP and total (T) fractions. Efficiency of the Ago2-IP procedure was confirmed by qRT-PCR for miR-378a-3p and miR-181a and by western blot for the Ago2 protein.

**Western blotting**

Infected cells were harvested and lysed in lysis buffer (#9803, Cell Signaling Technology, Danvers, Massachusetts, USA) supplemented with protease inhibitor. After centrifugation at 14,000 rpm for 10 minutes (4°C), supernatants were collected, and protein concentrations were measured using the BCA Protein Assay Kit (Thermo Fisher Scientific Inc.) according to the manufacturer’s protocol. 20 µg protein was
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Separated on a polyacrylamide gel and transferred to a nitrocellulose membrane, followed by incubation overnight at 4°C with primary antibodies diluted in 5% milk in Tris-buffered saline with Tween-20 (TBST) and anti-Ago2 (1000x diluted, 2E12-1C9, Abnova, Taipei, Taiwan). After incubation with the secondary antibody and ECL substrate (Thermo Fisher Scientific Inc.), chemiluminescence was detected with ChemiDoc MP Scanner and proteins were visualized and quantified with Image Lab 4.0.1 software (BioRad Hercules, CA, USA).

Microarray analysis

About 50 ng RNA of both the total (T) and the IP fractions were labeled and hybridized on an Agilent gene expression microarray (AMADID no.: 072363, SurePrint G3 Human Gene Exp v3 array kit, Agilent Technologies). The microarray contained 58,341 probes against coding and noncoding transcripts. The procedure and data analysis were performed as previously described [65]. Briefly, after cRNA synthesis and amplification, labeling was done with Cyanine 3-CTP (Cy3) or Cyanine 5-CTP (Cy5) using the LowInput QuickAmp Labeling kit (catalog no.: 0006322867). Equal amounts of Cy3- or Cy5-labeled cRNA samples were mixed and hybridized on the microarray slide overnight. Raw data were quantile normalized without baseline transformation using GeneSpring GX 12.5 software (Agilent Technologies). Probes were selected for further analysis if they were flagged present in all samples, expressed in the 25th to 100th percentile in at least half of the total (T) fractions, and showed consistent expression in the duplicate measurements (< 2-fold change). The average signals of replicates were used to calculate the IP/T ratio and probes with a ≥ 2-fold enrichment in the IP fraction as compared to total (T) fraction were considered as potential miRNA targets.

For pCDH-378a transduced cells, we next assessed miR-378a-3p targets by identifying probes that were enriched at least ≥ 2-fold higher in miR-378a overexpressing cells as compared to empty vector (pCDH-EV). For mZip-378a-3p transduced cells we assessed miR-378a-3p targets by identifying probes with at least ≥ 2-fold higher enrichment in mZip-SCR transduced cells as compared to mZip-378a-3p cells. Gene expression microarray data are deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo; accession number will follow soon).

Identification of miR-378a-3p seed sites and gene ontology (GO) terms

For all Ago2-RIP-Chip identified targets of miR-378a-3p, we used a Pearl script to search for 7mer-A1, 7mer-m8, and 8mer [71] miR-378a-3p seed sites in 5’-UTR, CDS, and 3’-UTR. Ensemble transcript isoforms were selected based on a refseq ID
conversion using Biomart (https://www.ensembl.org/biomart). For lncRNA transcripts without an Ensembl ID, we used the LNCipedia or a XLOC/TCONS (BROAD institute) transcript ID. The growth-related gene ontology (GO) terms (proliferation, cell cycle, and apoptosis) for selected genes were retrieved from the Ensembl database (https://www.ensembl.org/biomart).

Cloning of miRNA binding sites and luciferase reporter assay

We adapted the psi-Check-2 vector (Promega, Madison, WI, USA) to remove the predicted miR-378a-3p target site (7mer-A1) in the open reading frame of the Renilla luciferase gene. The binding site was mutated by changing two nucleotides in the seed region without affecting the amino acid sequence. This was accomplished by substituting the 460nt long fragment between the EcoRV and XhoI sites of the psi-Check-2 vector (Figure S6, Integrated DNA technologies, Leuven, Belgium). Effective Renilla luciferase production independent of miR-378a-3p levels was confirmed (data not shown) before cloning putative binding sites of target genes.

Ten potential miRNA binding sites of 8 miR-378a-3p target genes were ordered as 58-mer oligo duplexes (Integrated DNA technologies) and cloned into the XhoI and NotI restriction sites of the modified luciferase reporter vector (Table S3). For the binding sites with a positive result in the first luciferase reporter assay, mutant controls were generated by cloning oligo duplexes with mutations in 3 nucleotides in the seed region. The reporter vectors with miR-378a-3p wild type or mutated binding sites were co-transfected with 10 µM of either miR-378a pre-miRNA (Cat. NO.: AM17100, Ambion) or control oligos (Cat. NO.: AM17111, Ambion) to ST486 and DG75 cells using an Amaza nucleofector device (program A23) and the Amaza Cell Line Nucleofector Kit V (Cat NO.: VACA-1003) (Amaza, Gaithersburg, MD). Cells were harvested 24h after transfection. Renilla and Firefly luciferase activity in cell lysates were measured using a Dual-Luciferase Reporter Assay System (Promega). Each experiment was measured in duplicate and results were averaged per experiment. For each construct the luciferase assay was performed in three independent biological replicates.

Statistical analysis

MiRNAs significantly differentially expressed in the small RNA-seq profiling were identified with a moderated T-test and Benjamini-Hochberg correction for multiple testing using the GeneSpring GX software (version 12.5, Agilent Technologies Santa Clara, CA, USA). For confirmation of differentially expressed miRNAs by qRT-PCR, we used the nonparametric MannWhitney U-test (GraphPad Software Inc., San Diego, CA). Statistical analysis of GFP competition assays was performed as described previously [66]. Briefly, the percentage of mZip-378a-3p infected cells at day 4 was set

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MYC-induced miR-378a-3p is essential for BL growth to 100% and were compared to percentages in the control over time using a mixed model with time and the interaction of time and miRNA construct type as fixed effect and measurement repeat within miRNA construct type as random effect in SPSS (22.0.0.0 version, IBM, Armonk, New York, USA). For the luciferase reporter assay, significance was calculated based on the Renilla to Firefly (RL/FL) luciferase ratios between experimental samples and negative controls using paired t-test (GraphPad Software Inc.).

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**Conflicts of Interest:** The authors declare no conflicts of interest.

**Supplementary Figures**

**Figure S1.** Results of qRT-PCR validation experiments of the differential expression patterns observed by small RNA-seq in BL cell lines relative to germinal center B cells. The decreased levels of miR-28-3p and miR-30a-5p in BL cell lines relative to GC-B cells could not be validated by qRT-PCR. miRNA levels were normalized to RNU44.

**Figure S2.** MiR-378a-3p levels in BL and germinal center B cells based on small RNA-seq data.
BL cell lines (black bars) and germinal center B-cells (GC-B, white bars). RPM=reads per million.

Figure S3. Validation of Ago2-IP procedure in ST486 cells upon inhibition and overexpression of miR-378a-3p. Infection efficiency for (A) miR-378a-3p inhibition and (B) overexpression constructs in ST486. Enrichment of (C) miR-378a-3p and (D) a randomly selected control miRNA, miR-181a-5p in the Ago2 immunoprecipitated (IP) fraction in comparison to the total (T) fraction. (E) Western blot results of Ago2 protein in T, flow through (FT), and IP samples using the anti-Ago2 antibody or the control anti-IgG antibody for immunoprecipitation.
**Figure S4. Uncropped western blot results for Ago2 protein.** WB results of total (T), flow through (FT), and Ago2-immunoprecipitation (IP) fractions for ST486 cells using the anti-Ago2 antibody or the control anti-IgG antibody upon miR-378a-3p inhibition (A) and overexpression (B) experiments (see also Figure S3E). Arrows indicate the AGO2 protein. For each IP experiment the total fraction is set to 1.
Figure S5. Results of the luciferase reporter assays for 10 putative miR-378a-3p binding sites identified in 8 Ago-2 IP enriched genes. (A) results of ST486 and (B) DG75 cells. The 10 binding sites were cloned to the Psi-check-2 vector and co-transfected with miR-378a-3p mimics or negative control mimics to ST486 and DG75 BL cells. Binding of miR-378a-3p was confirmed for IRAK4, JPX, FOXP1-site1 and MNT in both cell lines.

Figure S6. Sequence of the minigene used to generate a luciferase reporter vector without the putative miR-378a-3p binding site in the Renilla gene. The miR-378a seed sequence is shown in green, with the mutated nucleotides in red.

```
5’ GATATCGCCCTGATCAAGAGCGAAGGGCGAGAAAATGGTGCTTGAGAATAACTTCTTCTCGAGA
CCATGCTCAGCAAGCAAGATCATAGCGAATACTGGAGCTTGAGGCTTGCTGCTCACTTGGAGCGAGTTCTGAGA
AGGAGAGGGGAGTTAGACGGCTCCCTCTTTCTTCCCGGCTCGAGATCCCTCTCGTTAAGGGAGCA
AGCCCGACGTCCGCAATTGTCGCAACTACAACCGCTCTTCCGGCGCCAGCGAGATTGCTGCTCATTAGA
TGTTGACTGAGTTAGCGCATATCCTGTGATGATTCTGGCAGGCTAAGAGAACGTTGCTGCTAGT
CCGAGTGCTGAGGCTGAGGGCGCCACTTCAGCCAGGAGAGCTCCAGATGAAATGGTGAAGTACA
TCAGAGCTGTTGAGCGCGTGTGAGAAGCGCAGTAATTCTAGCGATCGCTCGAG3’
```
## Supplementary Tables

### Table S1. Small RNA sequencing read summary

| Sample | Total after trimming Reads | Collapsed Reads | Mapping to Human genome Percentage | Collapsed Reads | Mapping to mirbase 21 Percentage | Collapsed Reads |
|--------|-----------------------------|-----------------|-------------------------------------|----------------|---------------------------------|-----------------|
| ST486  | 12,728,371                  | 679,193         | 9,215,947                           | 72.4%          | 477,577                        | 62.5%           |
| CA46   | 17,810,149                  | 193,500         | 15,429,749                          | 86.6%          | 45,386                         | 73.2%           |
| DG75   | 5,553,807                   | 295,796         | 4,542,877                           | 81.8%          | 192,986                        | 58.8%           |
| Ramos  | 9,001,167                   | 566,396         | 6,671,654                           | 74.1%          | 390,175                        | 44.9%           |
| GC-1   | 9,234,697                   | 357,071         | 6,993,299                           | 75.7%          | 176,567                        | 60.0%           |
| GC-2   | 9,637,128                   | 620,514         | 5,225,051                           | 54.2%          | 389,723                        | 26.3%           |
| GC-3   | 6,758,291                   | 524,000         | 4,660,685                           | 69.0%          | 284,438                        | 52.7%           |

### Table S2. Taqman miRNA assays used for qRT-PCR validation

| No. | miRNA    | Catalog No. | Sequence                  |
|-----|----------|-------------|---------------------------|
| 1   | miR-378a-3p | 2243        | 5’-ACUGGACUUGGAGUCAGAAGG-3’|
| 2   | miR-28-5p  | 0411        | 5’-AAGGAGCUCACAGUCUAUGAG-3’|
| 3   | miR-155-5p | 2623        | 5’-UUAAUGCUAAUCGUGAUAGGGU-3’|
| 4   | miR-363-3p | 1271        | 5’-AAUUGCAGCGUAUCCAUCUGA-3’|
| 5   | miR-222-3p | 2276        | 5’-AGCUACAUCUGGCUACUGG-3’    |
| 6   | miR-221-3p | 1134        | 5’-AGCUACAUCUGUCUGGUU-3’     |
| 7   | miR-30a-5p | 0417        | 5’-UGUAAACAUCCUCGAGUGGAAG-3’|
| 8   | miR-28-3p  | 2446        | 5’-CACUAGAUUGUAGCUCCUGGA-3’  |
Table S3. Oligos for cloning miR-378a-3p binding sites from selected genes, wild type and with mutations in miR-378a-3p seed.

| NO. | Gene   | Sense(S)/ Anti-sense(AS) | 5’ to 3’                  |
|-----|--------|--------------------------|---------------------------|
| 1   | FOXP1  | FOXP1-site1-MBS-S        | TCGAGAAGGGCCCTGTGCTTATGGAACACCACCCACTGAGAGGCCTCTC |
|     |        | FOXP1-site1-MBS-AS       | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | FOXP1-site1-MBS-mut-S    | GCACCTTTTATTTTCTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | FOXP1-site1-MBS-mut-AS   | GCCGAGGTTCCATGTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | FOXP1-site2-MBS-S        | TCGAGCAAATGGACATACCAAGCAGAGGGCCCTTC |
|     |        | FOXP1-site2-MBS-AS       | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
| 2   | IRAK4  | IRAK4-MBS-S              | TCGAATCTTGGACACACAGATGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | IRAK4-MBS-AS             | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | IRAK4-MBS-mut-S          | GCCGAGGTTCCATGTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | IRAK4-MBS-mut-AS         | GCCGAGGTTCCATGTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
| 3   | JPX    | JPX-MBS-S                | TCGAATCTTGGACACACAGATGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | JPX-MBS-AS               | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | JPX-MBS-mut-S            | GCCGAGGTTCCATGTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | JPX-MBS-mut-AS           | GCCGAGGTTCCATGTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
| 4   | MNT    | MNT-MBS-S                | TCGCCTCAAATACCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | MNT-MBS-AS               | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | MNT-MBS-mut-S            | GCCGAGGTTCCATGTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | MNT-MBS-mut-AS           | GCCGAGGTTCCATGTGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
| 5   | BCR    | BCR-site1-MBS-S          | TCGAATCTTGGACACACAGATGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | BCR-site1-MBS-AS         | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | BCR-site2-MBS-S          | TCGAATCTTGGACACACAGATGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | BCR-site2-MBS-AS         | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
| 6   | CISH   | CISH-MBS-S               | TCGAATCTTGGACACACAGATGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | CISH-MBS-AS              | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
| 7   | MYCBP  | MYCBP-MBS-S              | TCGAATCTTGGACACACAGATGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | MYCBP-MBS-AS             | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |
| 8   | TUBA1C | TUBA1C-MBS-S             | TCGAATCTTGGACACACAGATGAGTGCCTGCTGCTGACTAAGGACAGGGCCCTTC |
|     |        | TUBA1C-MBS-AS            | GCCCATGTCAAAATCTGACTTGCTGCTGCTGACTAAGGACAGGGCCCTTC |

Green letters indicate miR-378a-3p binding sites and red letters indicate the mismatches.
References

1. Burkitt, D.P. Etiology of Burkitt's lymphoma--an alternative hypothesis to a vectored virus. Journal of the National Cancer Institute 1969, 42, 19-28.
2. Zech, L.; Haglund, U.; Nilsson, K.; Klein, G. Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt lymphomas. International journal of cancer. 1976, 17, 47-56.
3. Victora, G.D.; Dominguez-Sola, D.; Holmes, A.B.; Deroubaix, S.; Dalla-Favera, R.; Nussenzweig, M.C. Identification of human germinal center light and dark zone cells and their relationship to human B-cell lymphomas. Blood 2012, 120, 2240-2248, doi:10.1182/blood-2012-03-415380.
4. Selbach, M.; Schwanhausser, B.; Thierfelder, N.; Fang, Z.; Khanin, R.; Rajewsky, N. Widespread changes in protein synthesis induced by microRNAs. Nature 2008, 455, 58-63, doi:10.1038/nature07228.
5. Baek, D.; Villen, J.; Shin, C.; Camargo, F.D.; Gygi, S.P.; Bartel, D.P. The impact of microRNAs on protein output. Nature 2008, 455, 64-71, doi:10.1038/nature07242.
6. Ameres, S.L.; Zamore, P.D. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013, 14, 475-488, doi:10.1038/nrm3611.
7. Lawrie, C.H. MicroRNAs in hematological malignancies. Blood reviews 2013, 27, 143-154, doi:10.1016/j.bbre.2013.04.002.
8. Oduor, C.I.; Kaymaz, Y.; Chelimo, K.; Otieno, J.A.; Ong'echa, J.M.; Moormann, A.M.; Bailey, J.A. Integrative microRNA and mRNA deep-sequencing expression profiling in endemic Burkitt lymphoma. BMC Cancer 2017, 17, 761, doi:10.1186/s12885-017-3711-9.
9. Robertus, J.L.; Klüiver, J.; Weggemans, C.; Harms, G.; Reijmers, R.M.; Swart, Y.; Kok, K.; Rosati, S.; Schuuring, E.; van Imhoff, G., et al. MiRNA profiling in B non-Hodgkin lymphoma: a MYC-related miRNA profile characterizes Burkitt lymphoma. Br J Haematol 2010, 149, 896-899, doi:10.1111/j.1365-2141.2010.08111.x.
10. Lenze, D.; Leoncini, L.; Hummel, M.; Volinia, S.; Liu, C.G.; Amato, T.; De Falco, G.; Githanga, J.; Horn, H.; Nyagol, J., et al. The different epidemiologic subtypes of Burkitt lymphoma share a homogenous micro RNA profile distinct from diffuse large B-cell lymphoma. Leukemia 2011, 25, 1869-1876, doi:10.1038/leu.2011.156.
11. Hezaveh, K.; Kloetgen, A.; Bernhart, S.H.; Mahapatra, K.D.; Lenze, D.; Richter, J.; Haake, A.; Bergmann, A.K.; Brors, B.; Burkhardt, B., et al. Alterations of microRNA and microRNA-regulated messenger RNA expression in germinal center B-cell lymphomas determined by integrative sequencing analysis. Haematologica 2016, 101, 1380-1389, doi:10.3324/haematol.2016.143891.
12. Di Lisio, L.; Sanchez-Beato, M.; Gomez-Lopez, G.; Rodriguez, M.E.; Montes-Moreno, S.; Mollejo, M.; Menarguez, J.; Martinez, M.A.; Alves, F.J.; Pisano, D.G., et al. MicroRNA signatures in B-cell lymphomas. Blood Cancer J 2012, 2, e57, doi:10.1038/bcj.2012.1.
13. Bartolome-Izquierdo, N.; de Yebeles, V.G.; Alvarez-Prado, A.F.; Mur, S.M.; Lopez Del Olmo, J.A.; Roa, S.; Vazquez, A.; van Imhoff, G., et al. miR-28 regulates the germinal center reaction and blocks tumor growth in preclinical models of non-Hodgkin lymphoma. Blood 2017, 129, 2408-2419, doi:10.1182/blood-2016-08-731166.
14. Chen, S.; Wang, Z.; Dai, X.; Pan, J.; Ge, J.; Han, X.; Wu, Z.; Zhou, X.; Zhao, T. Re-expression of microRNA-150 induces EBV-positive Burkitt lymphoma differentiation by modulating c-Myb in vitro. Cancer Sci 2013, 104, 826-834, doi:10.1111/cas.12156.
15. Dzikiewicz-Krawczyk, A.; Kok, K.; Slezak-Prochazka, I.; Robertus, J.L.; Bruining, J.; Tayari, M.M.; Rutgers, B.; de Jong, D.; Koerts, J.; Seitz, A., et al. ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma. Leukemia 2017, 31, 1470-1473, doi:10.1038/leu.2017.94.
16. Klüiver, J.; Haralambieva, E.; de Jong, D.; Blokzijl, T.; Jacobs, S.; Kroesen, B.J.; Poppema, S.; van den Berg, A. Lack of BIC and microRNA miR-155 expression in primary cases of
Burkitt lymphoma. Genes, chromosomes & cancer 2006, 45, 147-153, doi:10.1002/gcc.20273.

17. Sandhu, S.K.; Fassan, M.; Volinia, S.; Lovat, F.; Balatti, V.; Pekarsky, Y.; Croce, C.M. B-cell malignancies in microRNA Emu-miR-17-92 transgenic mice. Proceedings of the National Academy of Sciences of the United States of America 2013, 110, 18208-18213, doi:10.1073/pnas.1315351110.

18. Teng, G.; Hakimpour, P.; Landgraf, P.; Rice, A.; Tuschi, T.; Casellas, R.; Papavasiliou, F.N. MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. Immunity 2008, 28, 621-629, doi:10.1016/j.immuni.2008.03.015.

19. Dorsett, Y.; McBride, K.M.; Jankovic, M.; Gazumyan, A.; Thai, T.H.; Robbiani, D.F.; Di Virgilio, M.; San-Martin, B.R.; Heidkamp, G.; Schwickert, T.A., et al. MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc-Igh translocation. Immunity 2008, 28, 630-638, doi:10.1016/j.immuni.2008.04.002.

20. Feng, M.; Li, Z.; Aau, M.; Wong, C.H.; Yang, X.; Yu, Q. Myc/miR-378/TOB2/cyclin D1 functional module regulates oncogenic transformation. Oncogene 2011, 30, 2242-2251, doi:10.1038/onc.2010.602.

21. Pajic, A.; Spitkovsky, D.; Christoph, B.; Kempkes, B.; Schuhmacher, M.; Staeger, M.S.; Brielmeier, M.; Ellwart, J.; Kohlhuber, F.; Bornkamm, G.W., et al. Cell cycle activation by c-myc in a burkitt lymphoma model cell line. Int J Cancer 2000, 87, 787-793, doi:10.1002/1097-0215(20000915)87:6<787::aid-ijc4>3.0.co;2-6.

22. Li, H.; Dai, S.; Zhen, T.; Shi, H.; Zhang, F.; Yang, Y.; Kang, L.; Liang, Y.; Han, A. Clinical and biological significance of miR-378a-3p and miR-378a-5p in colorectal cancer. Eur J Cancer 2014, 50, 1207-1221, doi:10.1016/j.ejca.2013.12.010.

23. Megiorni, F.; Cialfi, S.; McDowell, H.P.; Felsani, A.; Camero, S.; Guffanti, A.; Pizer, B.; Clerico, A.; De Grazia, A.; Pizzuti, A., et al. Deep Sequencing the microRNA profile in rhabdomyosarcoma reveals down-regulation of miR-378 family members. BMC Cancer 2014, 14, 880, doi:10.1186/1471-2407-14-880.

24. Wang, M.; Sun, X.; Yang, Y.; Jiao, W. Long non-coding RNA OIP5-AS1 promotes proliferation of lung cancer cells and leads to poor prognosis by targeting miR-378a-3p. Thorac Cancer 2018, 9, 939-949, doi:10.1111/1759-7714.12767.

25. Chen, Q.G.; Zhou, W.; Han, T.; Du, S.Q.; Li, Z.H.; Zhang, Z.; Shan, G.Y.; Kong, C.Z. MiR-378 suppresses prostate cancer cell growth through downregulation of MAPK1 in vitro and in vivo. Tumor Biol 2016, 37, 2095-2103, doi:10.1007/s13277-015-3996-8.

26. Xu, Z.H.; Yao, T.Z.; Liu, W. miR-378a-3p sensitizes ovarian cancer cells to cisplatin through targeting MAPK1/GRB2. Biomedicine & Pharmacotherapy 2018, 107, 1410-1417, doi:10.1016/j.biopha.2018.08.132.

27. Liu, H.; Zhu, L.; Liu, B.; Yang, L.; Meng, X.; Zhang, W.; Ma, Y.; Xiao, H. Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer. Cancer Lett 2012, 316, 196-203, doi:10.1016/j.canlet.2011.10.034.

28. Yu, B.L.; Peng, X.H.; Zhao, F.P.; Liu, X.; Lu, J.; Wang, L.; Li, G.; Chen, H.H.; Li, X.P. MicroRNA-378 functions as an onco-miR in nasopharyngeal carcinoma by repressing TOB2 expression. Int J Oncol 2014, 44, 1215-1222, doi:10.3892/ijo.2014.2283.

29. Qian, J.; Lin, J.; Qian, W.; Ma, J.C.; Qian, S.X.; Li, Y.; Yang, J.; Li, J.Y.; Wang, C.Z.; Chai, H.Y., et al. Overexpression of miR-378 is frequent and may affect treatment outcomes in patients with acute myeloid leukemia. Leukemia Res 2013, 37, 765-768, doi:10.1016/j.leukres.2013.03.014.

30. Tanaka, H.; Hazama, S.; Iida, M.; Tsunedomi, R.; Takenouchi, H.; Nakajima, M.; Tokumitsu, Y.; Kanekiyo, S.; Shindo, Y.; Tomochika, S., et al. miR-125b-1 and miR-378a are predictive biomarkers for the efficacy of vaccine treatment against colorectal cancer. Cancer Sci 2017, 108, 2229-2238, doi:10.1111/cas.13390.

31. Vasilevsky, N.A.; Ruby, C.E.; Hurlin, P.J.; Weinberg, A.D. OX40 engagement stabilizes Mxd4 and Mnt protein levels in antigen-stimulated T cells leading to an increase in cell
MYC-induced miR-378a-3p is essential for BL growth

32. Campbell, K.J.; Vandenberg, C.J.; Anstee, N.S.; Hurlin, P.J.; Cory, S. Mnt modulates Myc-driven lymphomagenesis. Cell Death Differ 2017, 24, 2117-2126, doi:10.1038/cdd.2017.131.

33. Yang, G.; Hurlin, P.J. MNT and Emerging Concepts of MNT-MYC Antagonism. Genes (Basel) 2017, 8, doi:10.3390/genes8020083.

34. Hooker, C.W.; Hurlin, P.J. Of Myc and Mnt. J Cell Sci 2006, 119, 208-216, doi:10.1242/jcs.02815.

35. Hurlin, P.J.; Zhou, Z.Q.; Toyo-oka, K.; Ota, S.; Walker, W.L.; Hirotsune, S.; Wynshaw-Boris, A. Deletion of Mnt leads to disrupted cell cycle control and tumorigenesis. EMBO J 2003, 22, 4584-4596, doi:10.1093/emboj/cdg442.

36. Nilsson, J.A.; Maclean, K.H.; Keller, U.B.; Pendeville, H.; Baudino, T.A.; Cleveland, J.L. Mnt loss triggers Myc transcription targets, proliferation, apoptosis, and transformation. Mol Cell Biol 2004, 24, 1560-1569, doi:10.1128/mcb.24.4.1560-1569.2004.

37. Walker, W.; Zhou, Z.Q.; Ota, S.; Wynshaw-Boris, A.; Hurlin, P.J. Mnt-Max to Myc-Max complex switching regulates cell cycle entry. J Cell Biol 2005, 169, 405-413, doi:10.1083/jcb.200411013.

38. Kim, T.W.; Staschke, K.; Bulek, K.; Yao, J.; Peters, K.; Oh, K.H.; Vandenburg, Y.; Xiao, H.; Qian, W.; Hamilton, T., et al. A critical role for IRAK4 kinase activity in Toll-like receptor-mediated innate immunity. J Exp Med 2007, 204, 1025-1036, doi:10.1084/jem.20061825.

39. Kim, T.W.; Staschke, K.; Bulek, K.; Yao, J.H.; Peters, K.; Oh, K.H.; Vandenburg, Y.; Xiao, H.; Qian, W.; Hamilton, T., et al. A critical role for IRAK4 kinase activity in Toll-like receptor-mediated innate immunity. J Exp Med 2007, 204, 1025-1036, doi:10.1084/jem.20061825.

40. Kuppers, R. IRAK4 inhibition to shut down TLR signaling in autoimmunity and MyD88-dependent lymphomas. J Exp Med 2015, 212, 2184-2184.

41. Akhter, A.; Street, L.; Ghosh, S.; Burns, B.F.; Elyamany, G.; Shabani-Rad, M.T.; Stewart, D.A.; Mansoor, A. Concomitant high expression of Toll-like receptor (TLR) and B-cell receptor (BCR) signalling molecules has clinical implications in mantle cell lymphoma. Hematol Oncol 2017, 35, 79-86, doi:10.1002/hon.2251.

42. Akhter, A.; Masir, N.; Elyamany, G.; Phang, K.C.; Mahe, E.; Al-Zahrani, A.M.; Shabani-Rad, M.T.; Stewart, D.A.; Mansoor, A. Differential expression of Toll-like receptor (TLR) and B cell receptor (BCR) signalling molecules in primary diffuse large B-cell lymphoma of the central nervous system. J Neuro-Oncol 2015, 121, 289-296, doi:10.1007/s11060-014-1655-3.

43. Ngo, V.N.; Young, R.M.; Schmitz, R.; Jhavar, S.; Xiao, W.; Lim, K.H.; Kohlihammer, H.; Xu, W.; Yang, Y.; Zhao, H., et al. Oncogenically active MYD88 mutations in human lymphoma. Nature 2011, 470, 115-119, doi:10.1038/nature09671.

44. Kelly, P.N.; Romero, D.L.; Yang, Y.B.; Shaffer, A.L.; Chaudhary, D.; Robinson, S.; Miao, W.Y.; Rui, L.X.; Westlin, W.F.; Kapeller, R., et al. Selective interleukin-1 receptor-associated kinase 4 inhibitors for the treatment of autoimmune disorders and lymphoid malignancy. J Exp Med 2015, 212, 2189-2201, doi:10.1084/jem.20151074.

45. Klapproth, K.; Sander, S.; Marinkovic, D.; Baumann, B.; Wirth, T. The IKK2/NF-κB[p50]B pathway suppresses MYC-induced lymphomagenesis. Blood 2009, 114, 2448-2458, doi:10.1182/blood-2008-09-181008.

46. Gehringer, F.; Weissinger, S.E.; Moller, P.; Wirth, T.; Ushmorov, A. Physiological levels of the PTEN-PI3K-AKT axis activity are required for maintenance of Burkitt lymphoma. Leukemia 2019, 10.1038/s41375-019-0628-0, doi:10.1038/s41375-019-0628-0.

47. Coffer, P.J.; Burgering, B.M.T. Forkhead-box transcription factors and their role in the immune system. Nat Rev Immunol 2004, 4, 889-899, doi:10.1038/nri1488.
Chapter 3

49. Hu, H.; Wang, B.; Borde, M.; Nardone, J.; Maika, S.; Allred, L.; Tucker, P.W.; Rao, A. Foxp1 is an essential transcriptional regulator of B cell development. Nat Immunol 2006, 7, 819-826, doi:10.1038/nri1358.

50. Musilova, K.; Devan, J.; Cerna, K.; Seda, V.; Pavlasova, G.; Sharma, S.; Oppelt, J.; Pytlík, R.; Prochazka, V.; Prouzova, Z., et al. miR-150 downregulation contributes to the high-grade transformation of follicular lymphoma by upregulating FOXP1 levels. Blood 2018, 132, 2389-2400, doi:10.1182/blood-2018-06-855502.

51. Mottok, A.; Jurinovic, V.; Farinha, P.; Rosenwald, A.; Leich, E.; Ott, G.; Horn, H.; Klapper, W.; Boesl, M.; Hiddemann, W., et al. FOXP1 expression is a prognostic biomarker in follicular lymphoma treated with rituximab and chemotherapy. Blood 2018, 131, 226-235, doi:10.1182/blood-2017-08-799080.

52. van Keimpema, M.; Gruneberg, L.J.; Schilder-Tol, E.J.M.; Oud, M.E.C.M.; Beuling, E.A.; Hensbergen, P.J.; de Jong, J.; Pals, S.T.; Spaargaren, M. The small FOXP1 isoform predominantly expressed in activated B cell-like diffuse large B-cell lymphoma and full-length FOXP1 exert similar oncogenic and transcriptional activity in human B cells. Haematologica 2017, 102, 573-583, doi:10.3324/haematol.2016.156455.

53. Gascoyne, D.M.; Banham, A.H. The significance of FOXP1 in diffuse large B-cell lymphoma. Leukemia Lymphoma 2017, 58, 1037-1051, doi:10.1080/10428194.2016.1228932.

54. Cheng, L.; Shi, X.Z.; Huo, D.M.; Zhao, Y.; Zhang, H. MiR-449b-5p regulates cell proliferation, migration and radioresistance in cervical cancer by interacting with the transcription suppressor FOXP1. Eur J Pharmacol 2019, 856, doi:UNSP 17239910.1016/j.ejphar.2019.05.028.

55. Sun, X.; Wang, J.; Huang, M.; Chen, T.; Chen, J.; Zhang, F.; Zeng, H.; Xu, Z.; Ke, Y. STAT3 promotes tumour progression in glioma by inducing FOXP1 transcription. J Cell Mol Med 2018, 22, 5629-5638, doi:10.1111/jcmm.13837.

56. Wang, H.; Ding, Q.; Wang, M.; Guo, M.; Zhao, Q. miR-29b inhibits the progression of multiple myeloma through downregulating FOXP1. Hematology 2019, 24, 32-38, doi:10.1080/10245332.2018.1502961.

57. Sheng, H.; Li, X.Y.; Xu, Y. Knockdown of FOXP1 promotes the development of lung adenocarcinoma. Cancer Biology & Therapy 2019, 20, 537-545, doi:10.1080/15384047.2018.1537999.

58. Koon, H.B.; Ippolito, G.C.; Banham, A.H.; Tucker, P.W. FOXP1: a potential therapeutic target in cancer. Expert opinion on therapeutic targets 2007, 11, 955-965, doi:10.1517/14728222.11.7.955.

59. Sagardoy, A.; Martinez-Ferrandis, J.I.; Roa, S.; Bunting, K.L.; Aznar, M.A.; Elemento, O.; Shaknovich, R.; Fontan, L.; Fresquet, V.; Perez-Roger, I., et al. Downregulation of FOXP1 is required during germinal center B-cell function. Blood 2013, 121, 4311-4320, doi:10.1182/blood-2012-10-502061.

60. van Keimpema, M.; Gruneberg, L.J.; Mokry, M.; van Boxtel, R.; Koster, J.; Coffer, P.J.; Pals, S.T.; Spaargaren, M. FOXP1 directly represses transcription of proapoptotic genes and cooperates with NF-kappaB to promote survival of human B cells. Blood 2014, 124, 3431-3440, doi:10.1182/blood-2014-01-553412.

61. Tian, D.; Sun, S.; Lee, J.T. The Long Noncoding RNA, Jpx, Is a Molecular Switch for X Chromosome Inactivation. Cell 2010, 143, 390-403, doi:10.1016/j.cell.2010.09.049.

62. Li, J.; Feng, L.; Tian, C.; Tang, Y.L.; Tang, Y.; Hu, F.Q. Long noncoding RNA-JPX predicts the poor prognosis of ovarian cancer patients and promotes tumor cell proliferation, invasion and migration by the PI3K/Akt/mTOR signaling pathway. Eur Rev Med Pharmacol 2018, 22, 8135-8144.
interacting with miR-145-5p and CCND2. Carcinogenesis 2019, 10.1093/carcin/bgz125, doi:10.1093/carcin/bgz125.

64. Lin, X.Q.; Huang, Z.M.; Chen, X.; Wu, F.; Wu, W. XIST Induced by JPX Suppresses Hepatocellular Carcinoma by Sponging miR-155-5p. Yonsei Med J 2018, 59, 816-826, doi:10.1093/ymj/2018.59.7.816.

65. Winkle, M.; van den Berg, A.; Tayari, M.; Sietzema, J.; Terpstra, M.; Kortman, G.; de Jong, D.; Visser, L.; Diepstra, A.; Kok, K., et al. Long noncoding RNAs as a novel component of the Myc transcriptional network. Faseb J 2015, 29, 2338-2346, doi:10.1096/fj.14-263889.

66. Yuan, Y.; Kluiver, J.; Koerts, J.; de Jong, D.; Rutgers, B.; Razak, F.A.; Terpstra, M.; Plaat, B.E.; Nolte, I.M.; Diepstra, A., et al. miR-24-3p Is Overexpressed in Hodgkin Lymphoma and Protects Hodgkin and Reed-Sternberg Cells from Apoptosis. Am J Pathol 2017, 187, 1343-1355, doi:10.1016/j.ajpath.2017.02.016.

67. Friedlander, M.R.; Chen, W.; Adamidi, C.; Maaskola, J.; Einspanier, R.; Knespel, S.; Rajewsky, N. Discovering microRNAs from deep sequencing data using miRDeep. Nat Biotechnol 2008, 26, 407-415, doi:10.1038/nbt1394.

68. Griffiths-Jones, S.; Grocock, R.J.; van Dongen, S.; Bateman, A.; Enright, A.J. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic acids research 2006, 34, D140-144, doi:10.1093/nar/gkj112.

69. Kluiver, J.; Gibcus, J.H.; Hettinga, C.; Adema, A.; Richter, M.K.; Halsema, N.; Slezak-Prochazka, I.; Ding, Y.; Kroesen, B.J.; van den Berg, A. Rapid generation of microRNA sponges for microRNA inhibition. PLoS One 2012, 7, e29275, doi:10.1371/journal.pone.0029275.

70. Tan, L.P.; Seinen, E.; Duns, G.; de Jong, D.; Sibon, O.C.M.; Poppena, S.; Kroesen, B.J.; Kok, K.; van den Berg, A. A high throughput experimental approach to identify miRNA targets in human cells. Nucleic Acids Research 2009, 37, doi:ARTN e13710.1093/nar/gkp715.

71. Agarwal, V.; Bell, G.W.; Nam, J.W.; Bartel, D.P. Predicting effective microRNA target sites in mammalian mRNAs. eLife 2015, 4, doi:10.7554/eLife.05005.
