Blood and Lymphatic Cancer: Targets and Therapy

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REVIEW

Current understanding of the role and regulation of miRNAs in Burkitt lymphoma

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Abstract: Since its discovery in 1958, Burkitt lymphoma (BL) has been extensively studied and has become a model for tumorigenesis, but its pathogenesis has not been completely explained and understood yet. The aim of this review was to summarize the current knowledge about BL and, in particular, to discuss the role of miRNAs in its pathogenesis and their possible use as diagnostic and prognostic indicators. The impact of viral-encoded miRNAs is also discussed, with the Epstein–Barr infection being almost invariably detected in the endemic variant of this tumor.

Keywords: Burkitt lymphoma, miRNAs, *MYC*

Introduction

Discovery of Burkitt lymphoma (BL)

BL was first described by Denis Burkitt in 1958[1] during a field trip to sub-Saharan Africa and was subsequently named after him. The British surgeon observed that this particular tumor had a very high incidence in this geographic region, referred to as the "lymphoma belt" of Africa, in which other infectious diseases are also very common, such as malaria and arboviral infections.[2,3] Nevertheless, even though ~60 years have passed since the first description, it is not clear yet what impact, if any, these infectious diseases may have in driving BL in endemic areas.[4]

BL has been referred to as the "Rosetta stone" of cancer because it is the first tumor for which a viral association has been described (with the Epstein–Barr virus [EBV]),[5] the first tumor in which a specific chromosomal translocation has been identified (involving the *MYC* proto-oncogene)[6,7] and the first tumor successfully treated by chemotherapy.[8] For this reason, BL has a very important "historic" role, as it has improved our understanding of the molecular mechanisms happening in cancer, and it is still considered a model for tumorigenesis.

The EBV was isolated by Sir Anthony Epstein in 1964 from a BL-derived cell line,[5] and now clear evidence highlight that this virus is not simply a bystander, but it actively promotes transformation through its encoded genome products.[9]

Classification of BL

According to the World Health Organization classification, BL can be defined as a "single morphological and clinical entity, with variations in clinical presentation".[10] Three subtypes of this tumor have been described, namely, the endemic (eBL), the sporadic (sBL) and the immunodeficiency-associated form (ID-BL or human immunodeficiency

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virus [HIV]-BL) that differ in geographic distribution and the extent of association with viruses, with EBV being the most relevant.[10] Despite this distinction, these variants can still be considered the same entity as they all share the same clinical presentation and the same molecular profile,[11,12] with some differences in their miRNA profile.[13] The hallmark of BL is the constitutive activation of the *MYC* proto-oncogene, which leads to deregulated and increased expression of the Myc protein. In the vast majority of cases, this imbalance is achieved through a chromosomal translocation, which puts the *MYC* gene, mapping on chromosome 8, under the transcriptional control of immunoglobulin (Ig) gene promoters. When these promoters are very active, following a translocation, there is a strong and sustained expression of the *MYC* gene, with a resulting upregulation of its protein product.[6,7] Three different translocations have been described involving different Ig loci (t[8;14], t[8;2] and t[8;22]), of which the t(8;14) is the most frequently observed in BL (~80% of BL cases).[6,7] However, in the past 2 decades, it has been observed that BL cases overexpressing Myc, but lacking an identifiable translocation do exist.[14] These cases are indistinguishable from translocated BLs with respect to clinical presentation and share the same gene expression profile,[11] though they present with differences in their miRNA signature, as explained later in this review.[15] This suggests that alternative pathogenetic mechanisms responsible for increased levels of the Myc protein exist, besides the chromosomal translocation. Interestingly, no differences regarding the presence of *MYC* translocation are observed among the different clinical variants of BL. BL immunophenotype shows the expression of B-cell-associated antigens (eg, CD19, CD20 and CD22) and additional proteins such as CD10, BCL6, CD38, CD77 and CD43, which suggests its derivation from late germinal center (GC) cells.[10] However, it has been postulated that sBL and eBL derive from GC cells at different stages of differentiation, as they show a different pattern of Ig hypermutation and signs of antigen selection.[16] Based on this observation, it is possible that sBL derives from early GC cells (centroblasts), whereas eBL derives from late GC cells (centrocytes), as the latter show a higher number of somatic hypermutations in the Ig genes.[16]

Clinical variants of BL: endemic, sporadic and immunodeficiency associated

Despite BL showing a very homogeneous molecular profile,[11,12] differences can be pinpointed regarding the geographic distribution of this tumor and association with EBV. The endemic form has a very high incidence in Equatorial Africa, where other climatic conditions and infectious agents may possibly act as cofactors, though it is still debated at which extent and through which mechanisms.[4] The endemic form of BL is preferentially observed in young children (especially males), with a peak incidence of 4–7 years. It most commonly presents in extranodal sites, with the jaw and other facial bones being very frequently affected. Most importantly, the degree of association of this clinical variant with EBV is extremely high, the virus being detected in ~100% of eBL cases.[10] Such a strong association suggests an active involvement of EBV in Burkitt pathogenesis, and recent literature proves an important role for its encoded products,[9] although there is still a lot to uncover. Based on the simple association with EBV, 90–95% of the world population test positive for EBV, but BL incidence is much lower. This suggests that other factors may be required for BL pathogenesis.

The sporadic form occurs anywhere in the world and is histologically identical to eBL. It is still a pediatric disease, accounting for ~30% of childhood lymphomas (and 1%–2% of all lymphomas), and yet has a higher incidence in males, though with a higher median age of incidence (12 years). Another difference is that, despite sBL also showing an extranodal presentation, jaw tumors are less frequent, with the gastrointestinal tract being the most common site of involvement. Very interestingly, EBV is less commonly associated with sBL, being detected only in ~20%–30% of cases.[10]

As far as the immunodeficiency-related form is concerned, this clinical variant is particularly frequent in HIV-positive individuals, accounting for one-third of HIV-associated lymphomas, and it is therefore also referred to as HIV-related BL. This tumor is mostly observed in adults; it mainly shows a nodal presentation, with a generalized lymph node involvement and the bone marrow and central nervous system involvement also being common. Extranodal disease is possible, but it is much less frequent than in endemic and sporadic forms. The immunodeficiency-related variant shows a variable extent of association with EBV, ranging from 30% to 90% of all cases.[10] Noteworthy, this form is not only observed in HIV-infected patients but also in other types of immunodeficiency (ie, posttransplant), although BL may be the first disease to manifest in HIV-infected individuals, as it occurs in patients with a still high $CD4^+$ count and not having overt acquired immunodeficiency syndrome yet. This evidence suggests that, despite its role as an oncogenic virus still being debated, HIV may actively contribute to BL pathogenesis either indirectly, through a

continuous antigenic stimulation, or directly, through its encoded genome products.[17]

MYC deregulation in BL

The *MYC* proto-oncogene is a powerful transcription factor and plays very important physiological functions, being involved in the control of proliferation, cell growth, metabolism, apoptosis and differentiation.[18] It belongs to the Myc family of transcription factors (comprising *MYC*, *MYCN* and *MYCL*), of which *MYC* is the best characterized. It was originally identified because of its homology with *v-MYC*, the transforming gene of the MC29 avian leukemia virus,[19] and subsequently, its deregulation has been reported in a wide range of human tumors, though its activation may be achieved through different pathogenetic mechanisms.[20] *MYC* may either induce or repress transcriptional activation, thus regulating the expression of many downstream targets and consequent biological pathways.[21] To carry out transcriptional control, the Myc protein binds to other transcriptional regulators such as Max or Mnt, Mxd1-4 (Mad1, Mxi1, Mad3 and Mad4) and Mga, which influence Myc transcriptional regulation pushing toward either activation or repression of downstream targets. Proliferative stimuli induce the expression of *MYC* and lead to the formation of Myc:Max heterodimers and concomitant activation of target gene expression, thus resulting in transcriptional activation.[21] Other transcription factors may compete with Max for binding to Myc (Mnt, Mxd1-4 and Mga), thus resulting in transcriptional repression.[22] In addition, Myc can bind to transcription factors Sp1 and Miz1 and may interfere with their transcriptional activator capability. The complex Myc–Miz1 recruits DNA methyl transferase 3a and histone deacetylase 3 to gene promoters, leading to DNA cytosine methylation and histone deacetylation, therefore causing gene expression silencing. The Myc–Miz1 complex can, therefore, induce the formation of heterochromatin on its target sites and function as a transcriptional repressor complex.[22] Due to its key involvement in transcription regulation, Myc expression and function must be tightly controlled.

Pathological activation of *MYC* associated with gain-of-function mutations has been commonly described in cancer. It can be due to chromosomal translocations leading to promoter rearrangements (as observed in most BL cases), gene amplifications (commonly reported in breast cancer), virus-mediated insertional mutagenesis (less commonly observed, due to random insertions of viruses within the genome) and Myc protein stabilization, mainly due to genetic mutations.[21] Though all these mechanisms are possible in BL, the most commonly observed cause of Myc deregulation is usually the presence of a balanced translocation involving chromosome 8, where *MYC* maps, and different partners. Mutations of the *MYC* coding sequence have also been described in BL, as well as variations in its copy number, but they occur in a minority of cases[23] and do not seem to account for the main reason of *MYC* upregulation. Nevertheless, in the last few years, a few cases of BL in which none of the abovementioned mechanisms could possibly explain Myc hyperexpression have been described, and alternative pathogenetic mechanisms were investigated. No matter what is leading to *MYC* deregulation, the consequence is an increased expression of its protein product which is invariably associated with genomic instability, uncontrolled cell proliferation, escape from immune surveillance and malignant transformation.[20]

Additional genetic lesions in BL

Despite *MYC* deregulation being absolutely crucial for BL pathogenesis, *MYC* imbalance is not the only genetic lesion identified in BL and other recurrent or sporadic lesions have also been described. Several genes have been reported to be mutated in BL, such as the tumor suppressors *ID3*[24,25] or *TCF3*,[26] whose mutations seem to be quite common in BL, especially in its sporadic variant. Additional mutations have been detected in genes belonging to the PI3K pathway,[27] in the SWI/SNF family members and in *ARID1A* and *SMARCA4A* among others, which suggest functional alterations of the nucleosome remodeling complex.[28] In addition, genes whose mutations have already been described in other B-cell lymphomas, such as *MYC* itself, *DDX3X*, *CCND3* and *FBXO11*, among others, have also been reported to be mutated in BL.[25,26] Recently, mutations of particular genes have been reported to occur at a different frequency in eBL and sBL, and a correlation between a distinct mutation pattern and the existence of some viral infections has been suggested.[29] This observation may indicate that different pathogenetic mechanisms may exist in eBL and sBL and again suggests that viruses may play an active role in contributing to BL development.

Genetic lesions other than point mutations have also been described in BL. In particular, copy number alterations including gains of 1q, 9q, 12q, 13q, 20q, 22q and Xq and losses of 4q, 13q and 17p have been reported.[25,30–34] Additionally, trisomy 1q[31] and tetrasomy 1q have also been described.[32] Interestingly, gains of 11q have been frequently reported in a subset of tumors resembling BL but lacking *MYC* translocation,[35] although it is debated whether such cases should be diagnosed as BLs or rather as different aggressive B-cell tumors with a Burkitt-like presentation.[35]

In addition, uniparental disomy, whose role has been recently highlighted in cancer,[36] does not seem to play a major role in the pathogenesis of BL.[37]

Myc upregulation due to impairment of post-transcriptional regulation: the role of miRNAs

Regulation of gene expression must be finely tuned and may be controlled at different levels. Transcriptional regulation can be achieved by epigenetic changes, which regulate the accessibility of specific DNA sequences through methylation of histones and DNA, thus determining a conformational change in the chromatin and preventing the expression of genes when their function is not required. Regulation of gene expression is further controlled at the post-transcriptional level, when a certain mRNA has already been transcribed, but translation into the correspondent protein product is impaired. Post-transcriptional regulation is achieved by small non-coding RNAs, of which miRNAs have been intensively studied in the last few years.

miRNAs were isolated for the first time from *Caenorhabditis elegans* in 1998,[38] and since the first observation, they have been described in a wide range of organisms, including humans. They are small sequences of non-coding RNA that, in their mature form, have sizes of 18–24 bp, though they are processed from longer precursors during a maturation process that also shuttles them from the nucleus to the cytoplasm.[38] After maturation, the miRNAs bind to complementary mRNA sequences and prevent their translation into the corresponding proteins. Depending on their degree of complementarity with the target mRNAs, they can either lead to mRNA degradation, when there is a perfect pairing, or simply to translation impairment, if there are mismatches in the pairing. However, no matter whether the mRNA is degraded or not, the consequence of miRNA–mRNA binding is that production of the protein coded by that particular mRNA is prevented. This further level of regulation allows shutting down the expression of specific genes even when their transcription into an mRNA has already taken place. More importantly, it is worth mentioning that a single miRNA can target hundreds of mRNAs based on a short sequence complementarity. Deregulation of a single miRNA may, therefore, result in deregulated expression of many genes, thus affecting several distinct pathways and biological functions within the cell. With the function of miRNA being so delicate in tuning the gene expression, their function must be strictly controlled as its imbalance might lead to disturbance of gene expression. Deregulation of miRNA expression and function has, therefore, been reported in a plethora of human diseases, including cancer.[39]

MYC and miRNAs control each other's expression: the existence of a feedback regulatory loop

We have already mentioned the importance of *MYC* as a transcription regulator. With its capability to bind to target sequences on DNA, Myc can control the expression of coding as well as non-coding regions in the DNA, including genes and miRNAs. Myc is known to regulate the expression of ~60 miRNAs,[40,41] either positively or negatively influencing their expression (Figure 1). As a transcriptional activator, Myc can induce the expression of selected miRNAs, of which the miR-17-92 cluster is the prototypical example,[42] thus influencing the expression of miRNA downstream target genes and eventually influencing the related biological processes (Figure 2A). Induction of miR-17-92 by *MYC* has been previously reported not only in BL and other B-cell tumors[42] but also in various different tumors including breast, lung, colon, stomach and prostate (for a review, see Bui and Mendell[43]). Inhibition of downstream target genes of the miR-17-92 cluster enhances tumorigenicity by boosting cell proliferation, tumor cell survival and angiogenesis, along with metabolic reprogramming.[43] Very interestingly, among the downstream targets of this cluster, there are tumor suppressors such as *PTEN* and *BIM*, the first gene being an antagonist of PI3K activity and the latter having a proapoptotic function.[43–46] Overexpression of miR-17-92 induced by Myc, therefore, results in loss of regulatory control on cell growth mediated by these tumor suppressors. A recent study investigated the expression of each member of this cluster in BL and analyzed whether there was a correlation between their expression and prognosis of BL.[47] The results of this study indicated that miR-17 and miR-20a were highly expressed in BL and determined lack of expression of the Bim protein.[47] In addition, a significant correlation between high levels of miR-17 and poor overall survival was also recorded, thus indicating the influence of miRNA expression as a prognostic value in BL.[47]

At the same time, Myc can also repress the expression of specific miRNAs (ie, the miR-29 family), thus leading to increased expression of miRNA target genes and imbalance of cellular pathways (Figure 2A). However, *MYC* itself is a gene and, therefore, its expression is also controlled at the post-transcriptional level by miRNAs, of which probably the best studied is the let-7 family.[48] *MYC* expression, both at the transcriptional and post-transcriptional levels, is therefore

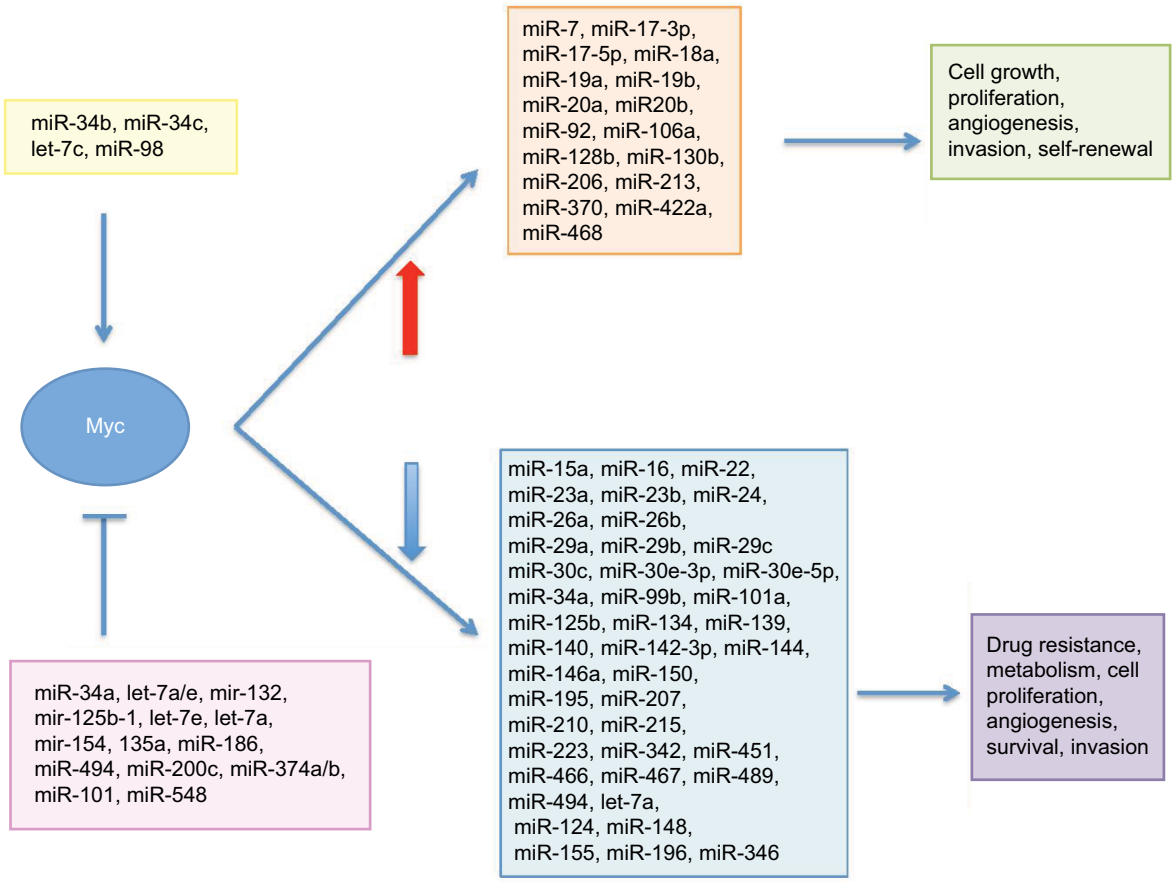

Figure 1 An overview of *MYC*–miRNA regulatory loop and related pathways.
Note: miRNAs upregulated by Myc are indicated by the red arrow and the downregulated miRNAs are indicated by the blue arrow.

controlled through different mechanisms to ensure that this protein will be produced only when its function in the cell is needed. Thus, miRNAs–*MYC* form a feedback regulatory loop controlling each other's expression in an inverse and reciprocal manner (Figure 1). When this miRNAs–*MYC* autoregulation fails, the expression of *MYC* and miRNAs is no longer regulated and may result in diseases and cancer.

A recent study reported the upregulation of the *YY1* gene, which is an oncogenic transcription factor able to induce *MYC* expression, in BL as a consequence of downregulation of specific miRNAs.[49] This transcription factor was previously found to be upregulated in other non-Hodgkin's lymphomas (NHLs)[50,51] and plays a role in resistance to chemotherapy and immunotherapy in NHL cell lines.[52] *YY1* can also act as a transcriptional repressor of tumor suppressors such as p16, p27, p73 and p53.[53–55] In particular, its inhibitory effect on p53 is related to evasion from apoptosis,[56] which may be crucial for transformed cells, pointing at *YY1* as an indicator of aggressiveness in NHLs. Very interestingly, upregulation of *YY1* reported in this study is a consequence of repression of specific miRNAs, some of which, such as has-miR-363 and hsa-miR-200a, are among the top 20 miRNAs repressed in BL, thus reinforcing its functional role in the pathogenesis of BL.[49]

Exploring the *MYC*–miRNA interaction in BL: sustaining Myc hyperexpression in the absence of a translocation

Approximately 10% of BL cases lack an identifiable *MYC* translocation, but do express the Myc protein at a level comparable to *MYC*-translocated BL cases.[57] This observation prompted many scientists worldwide to explore alternative pathogenetic mechanisms that could explain a higher expression of Myc in the absence of genetic lesions, either translocation or copy number alterations. Given the functional relationship between *MYC* and miRNAs, one possible scenario to explore was to investigate whether there was an imbalance in *MYC*-regulating miRNA expression that could eventually explain increased Myc protein levels. A pioneer study published in 2008 compared the expression of six miRNAs predicted to target *MYC* (hsa-miR-155, has-miR-30a-3p, hsa-miR-34b, hsa-let-7c, hsa-let-7a and hsa-miR-98) between BL cases carrying or not an *MYC* translocation.[57] The results of

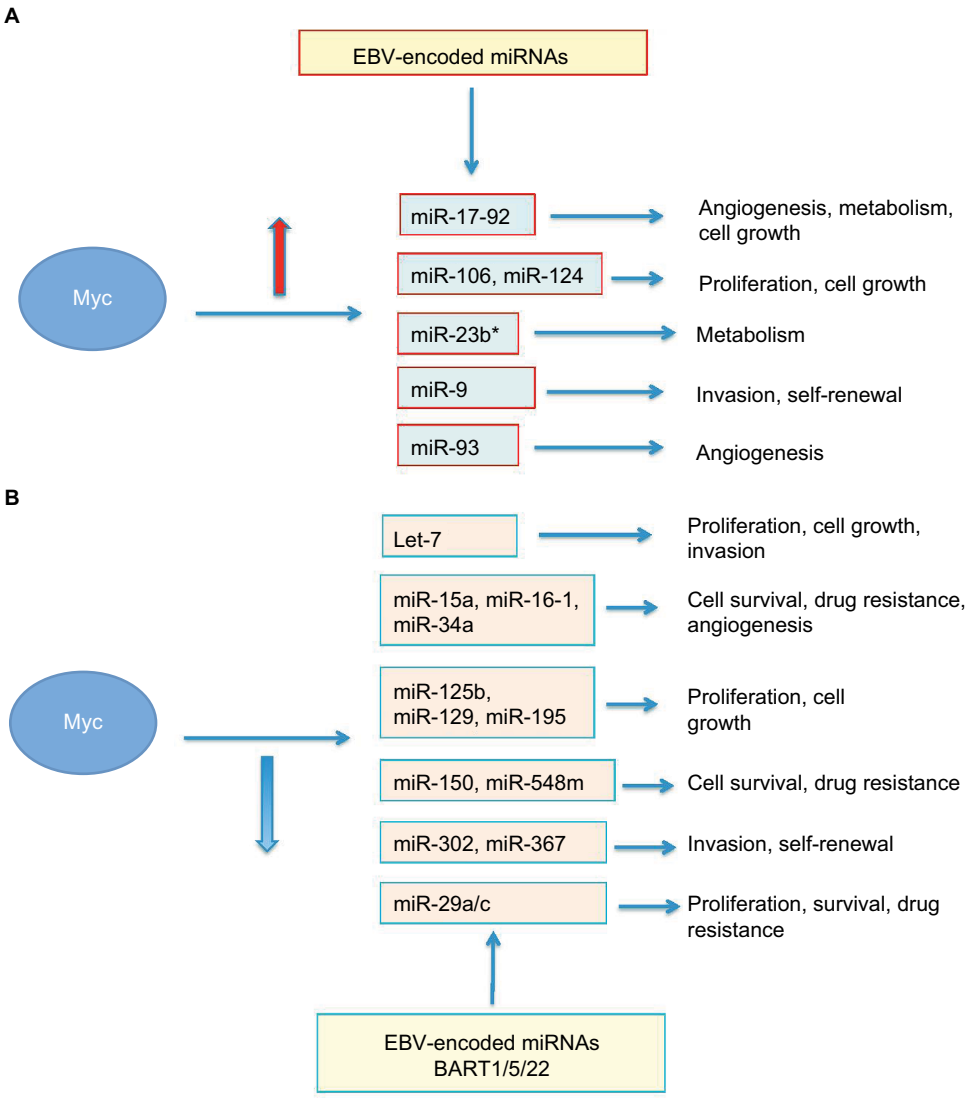

Figure 2 An overview of the pathways affected by Myc-regulated miRNAs.
Notes: (A) Myc-induced miRNAs and their regulated pathways. EBV-encoded miRNAs may compete with the miR-17-92 cluster for regulation of the same target genes. (**B**) Myc-repressed miRNAs and their regulated pathways. EBV-encoded BARTs may compete with cellular miR-29 family members.
Abbreviation: EBV, Epstein–Barr virus.

this study highlighted a diminished expression of two of them (hsa-let-7a and hsa-miR-34b), thus suggesting that increased expression of Myc may be a consequence of downregulation of specific miRNAs.[57] However, of even greater interest was the observation that hsa-miR-34b was downregulated only in BL cases lacking the translocation, whereas reduced expression of hsa-let-7a was observed in all BL cases, irrespective of the translocation status. This observation suggested the alteration of hsa-miR-34b as potentially responsible for Myc hyperexpression in the absence of any genetic lesions.[57] A later study of the same group identified hsa-miR-9* as a second miRNA specifically downregulated only in BL cases lacking *MYC* translocation.[58] This observation was of particular interest because hsa-miR-9* does not directly target *MYC*, but may indirectly regulate its expression through E2F1, whose expression is induced by Myc,[59,60] and that in turn activates *MYC* expression through a feedback autoregulatory loop that also involves the miR-17-92 cluster.[61–63] Hsa-miR-9* downregulation observed in BL cases lacking *MYC* translocation could determine the upregulation of E2F1, which then increases and sustains *MYC* expression. The complete miRNA expression profile was then investigated in BL cases with or without *MYC* translocation and differential expression of four miRNAs (hsa-miR-29a, hsa-miR-29b, hsa-miR-513a-5p and hsa-miR-628-3p) was identified.[15] A single miRNA is able to control the expression of many target genes. Therefore, we investigated the impact of the 4 dysregulated miRNAs so-identified on the global gene expression and identified 64 putative target genes

of such miRNAs were identified by bioinformatics.[15] The 64 predicted target genes are involved in important biological processes such as gene expression regulation, proliferation and DNA modification. Very interestingly, among differentially expressed genes, some, such as *MYCN* and the DNMT family of proteins, were of particular interest, with *MYCN* being a homologue of *MYC* and the DNMT proteins being reported to be altered in cancer very frequently.[64] DNMTs were reported to be specifically upregulated in BL cases lacking the translocation, suggesting that an aberrant epigenetic control occurs in this set of BL cases. As discussed earlier, interaction of Myc with DNMT family members can influence chromatin conformation and subsequent accessibility to RNA polymerase for transcription.[22] Deregulation of DNMTs by the miR-29 family has also been recently described in another study, which highlights the importance of epigenetic regulation in BL.[65] In particular, this study shows hypermethylation of p16 following overexpression of DNMTs, which might favor cell proliferation due to lack of control on cell cycle.[65] Of great interest is the finding that *MYCN* overexpression occurs only in cases lacking *MYC* translocation. *MYCN* expression is usually not detected in BL cases, but its deregulation is frequently observed in other cancers, such as neuroblastoma, where there is a different genetic mechanism (amplification of the *MYC* gene) which is responsible for over-expression of the Myc protein.[66] High expression of *MYCN* in cases lacking *MYC* translocation may indicate the existence of an alternative cooperative mechanism ensuring high expression of members of the *MYC* family in the absence of genetic lesions involving *MYC*. Very interestingly, deregulation of two of the differentially expressed miRNAs (miR-513a-5p and miR-628-3p) has been recently described in human neuroblastoma,[67,68] with the miR-628-3p expression correlating with the prognosis of this tumor.[69]

MYC pathway in BL: downregulated miRNAs target genes belonging to the *MYC* pathway

Several genes are transcriptionally controlled by Myc, and the existence of an *MYC* pathway has been identified in cells. As Myc is highly expressed in BL, it was investigated to what extent the Myc-regulated pathway was affected in BL and through which mechanisms. Results from a previous research study report that *MYC* target genes are upregulated in BL or gamma-irradiated mice tumors.[70] In this study, 41 miRNAs were found to be downregulated in gamma-irradiated mice lymphomas and 17 miRNAs in BL, resulting in upregulation of miRNA target genes. Interestingly, an enrichment of the *MYC* pathway was observed among upregulated genes, thus suggesting that upregulation of *MYC* pathway may be a consequence of transcriptional repression of specific miRNAs.[70]

miRNA expression profile: a BL signature for differential diagnosis

BL is a very homogeneous entity in terms of gene expression and has a distinctive, unique pattern, which distinguishes it from any other B-cell lymphomas.[11,12] This observation is extremely useful for diagnostic purposes, as we can classify borderline cases or cases with a histologic "Burkitt-like" presentation based on their distinctive molecular profile.[11,12] Despite showing some subtle differences in their miRNA profile, only a few differences can be identified at the gene expression level in cases with or without *MYC* translocation after enrichment, yet indicating a very high homogeneity of these tumors. The three clinical forms of BL share the same molecular signature, although differences in gene expression can be observed between EBV^+ and EBV^- BL cases.[71] However, such variations may be attributed to the presence of the virus rather than to differences between clinical variants.[71] The miRNA profile of BL was analyzed and compared to that of diffuse large B-cell lymphoma (DLBCL); a signature of 38 miRNAs was identified, which comprises *MYC*-regulated and nuclear factor-kB-associated miRNAs.[13] The same study also reported that only six miRNAs were differentially expressed between eBL and sBL, thus reinforcing the notion that BL is a very homogeneous entity and its molecular uniqueness can be used for differential diagnosis with other B-cell lymphomas.[13]

More recently, additional studies have confirmed the efficacy of miRNA profile for differential diagnosis and have proved that its reliability can be comparable to gene expression profile results, so far considered the "gold standard" for molecular analyses. A recent study identified a 27-miRNA signature able to distinguish BL from DLBCL, which could also be validated in formalin-fixed paraffin-embedded cases, thus even reinforcing its possible diagnostic application.[72] Another research identified by deep sequencing the existence of a 22-miRNA signature, which could be used to discriminate BL from DLBCL and follicular lymphomas, again highlighting the importance of molecular profiles for differential diagnosis.[73]

Noteworthy, low or no expression of hsa-miR-155 (or its precursor *BIC*) was detected in BL, as reported by several studies,[74–76] despite this miRNA being one of the most commonly upregulated in B-cell lymphomas.[77–79] It has been recently demonstrated that low levels of this miRNA determine an increased expression of *AICDA*, which increases the frequency of *MYC* translocation.[80] Intriguingly, a study shows that higher expression of miR-155 can be found in a

subset of EBV-positive BL cell lines expressing a viral latency III program, which is usually not detected in primary BLs, whereas low or no expression of this miRNA was detected in EBV-positive BL cases expressing a latency I program, which represent the vast majority of BLs.[75] It has sometimes been speculated that EBV infection may promote *MYC* translocation in BL. This observation may suggest a possible mechanism leading to *MYC* translocation in EBV-positive cases by maintaining low miR-155 and consequently inducing *AICDA* expression. Interestingly, downregulation of this miRNA can also be used for differential diagnosis,[81] as high expression of miR-155 is normally reported in other B-cell tumors, where it seems to have a clinical significance as it is associated with chemotherapy failure in DLBCL.[72]

Tables 1 and 2 list the miRNAs whose expression has been reported to be unbalanced in BL.

Virus-encoded miRNAs and deregulation of host cell gene expression: contribution of EBV

EBV is very often associated with BL, especially in its endemic form. Despite an extensive discussion about this virus, with its latency programs and pathogenetic mechanisms being beyond the aim of this review, it is worth mentioning that EBV may contribute to the pathogenesis of BL through its encoded proteins (genes and viral miRNAs). In BL cells, EBV expresses a latency I program, in which EBNA1 is the only viral protein expressed. Previous studies have demonstrated the role of this protein in the pathogenesis of BL.[82,83] EBV also encodes for 44 mature viral miRNAs (viRNAs) belonging to two families (BART and BHRF).[84] Expression of these viral-encoded miRNAs is also latency regulated, and only a few viRNAs belonging to the BART family are detected in BL.[85] It has also been demonstrated that three members of the BART family (BART-1-3p, BART-5-5p and BART-22-3p) exhibit high similarity with cellular miRNAs, including the miR-29 family (Figure 2B).[86–91] The expression of viRNAs should be carefully monitored as they compete with cellular miRNAs for the same target genes in the host cell. An interesting study has shown that in a BL-derived cell line expressing a latency III program, EBV-encoded miRNAs target the same genes as the miR-17-92 cluster (Figure 2A).[92] Despite the BL primary tumors mostly expressing a latency I program, this observation is of

Table 1 List of miRNAs reported to be downregulated in BL

Downregulated miRNAs	References
hsa-miR-221, hsa-miR-155, hsa-miR-146a, hsa-miR-146b-5p, hsa-miR-26b, hsa-miR-23a, hsa-miR-30d, hsa-miR-107, hsa-miR-103, hsa-miR-222, hsa-miR-26a, hsa-miR-30a, hsa-miR-142-5p, hsa-miR-23b, hsa-miR-342-3p, hsa-miR-29b, hsa-miR-34b	13
hsa-let-7a, hsa-miR-34b	57
hsa-miR-9*	58
hsa-miR-29a, hsa-miR-29b	15
hsa-155, hsa-196b, hsa-885-5p, hsa-222, hsa-135b, hsa-21, hsa-31, hsa-708, hsa-23a, hsa-455-5p, hsa-455-3p, hsa-29b, hsa-29c, hsa-342-5p, hsa-146a, hsa-150	72
hsa-513, hsa-18b, hsa-15b, hsa-454-3p, hsa-148a, let-7f, hsa-98, hsa-363, hsa-582, hsa-146, hsa-155, let-7d, hsa-26b, hsa-29b, hsa-142-3p, hsa-16, hsa-15a, hsa-590, hsa-32, hsa-331, hsa-138, hsa-28	70
hsa-miR-664-3p, hsa-miR-664-5p, hsa-miR-150-3p, hsa-miR-150-5p, hsa-miR-155-5p, hsa-miR-184, hsa-miR-196b-5p, hsa-miR-151b, hsa-miR-211-5p, hsa-miR-221-3p, hsa-miR-29c-5p	73
hsa-miR-155	74–76

Abbreviation: BL, Burkitt lymphoma.

Table 2 List of miRNAs reported to be upregulated in BL

Upregulated miRNAs	References
hsa-miR-371-5p, hsa-miR-185, hsa-miR-93*, hsa-miR-326, hsa-miR-497, hsa-miR-26b*, hsa-miR-339-5p, hsa-miR-485-3p, hsa-miR-9, hsa-miR-193a-5p, hsa-miR-448, hsa-miR-202*, hsa-miR-483-3p, hsa-miR-26a-1*, hsa-miR-328, hsa-miR-192, hsa-miR-429, hsa-miR-324-5p, hsa-miR-340, hsa-miR-105*, hsa-miR-124*	13
hsa-miR-17-92	42
hsa-miR-513a-5p, hsa-miR-628-3p	15
hsa-296-5p, hsa-296-3p, hsa-130b, hsa-18a, hsa-18b, hsa-19a, hsa-19b, hsa-20a, hsa-20b, hsa-17, hsa-106	72
hsa-202, hsa-92, hsa-19a, hsa-19b, hsa-296, hsa-663, hsa-22, hsa-320, hsa-181b, hsa-422b, hsa-484	70
hsa-miR-17-3p, hsa-miR-18a-3p, hsa-miR-19a-3p, hsa-miR-20a-3p, hsa-miR-25-5p, hsa-miR-93-3p, hsa-miR-106b-3p, hsa-miR-106b-5p, hsa-miR-130b-3p, hsa-miR-296-3p, hsa-miR-335-3p, hsa-miR-339-5p, hsa-miR-573, hsa-miR-4521	73
hsa-miR-21, hsa-miR-23a	100

Abbreviation: BL, Burkitt lymphoma.

interest as it highlights how EBV infection may contribute to deregulate key cellular pathways such as transcription, apoptosis and cell cycle.[92] Expression of viRNAs could, therefore, result in an aberrant post-transcriptional regulation in infected cells. The presence of EBV can impact on cellular miRNA signature in BL[85,93] and on cellular gene expression profile.[93] An important role for EBV-Bart6 has been suggested, as this miRNA is capable of regulating the expression of *PTEN* and interleukin-6 receptor complex in infected cells, thus influencing survival and interleukin-6 downstream pathways.[85] A later study confirmed this finding and highlighted the existence of a synergistic effect between Bart-6 and miR-142, which was previously reported to be upregulated in BL,[85] to repress *PTEN*.[94] This latter observation is of interest as it highlights the active role of EBV in BL pathogenesis and its interplay with the cellular machinery. It has also been demonstrated that BARTs target *Casp3* in BL and may therefore result in an antiapoptotic effect, thus resulting in a growth advantage for the infected cells.[95]

It is worth mentioning that EBV infection contributes to lymphomagenesis also through mechanisms other than miRNA regulation. We had previously mentioned that a different mutational profile can be observed between eBL and sBL, with endemic cases showing a lower mutation rate.[29] With the degree of association with EBV being the main difference between the endemic and sporadic variants, it is reasonable to postulate that such difference may be due to the existence of additional pathogenetic mechanisms in EBV-positive cases.[29] In a recent research paper, the methylome of EBV-positive vs EBV-negative BL-derived cell lines was compared, and the results of this study demonstrated that the presence of the virus is associated with a specific pattern of DNA methylation, suggesting that EBV may contribute to BL pathogenesis through an epigenetic mechanism.[96] In particular, this paper has demonstrated a higher level of methylation in EBV-positive samples involving, among others, key genes such as *ID3* and *TCF3* which are usually mutated in sBL, but whose mutation rate is lower in eBL.[29] Diminished expression of these genes in eBL may, therefore, be a consequence of epigenetic regulation rather than deriving from genetic lesions. This finding suggests that BLs have similar gene expression patterns, but underlying mechanisms may be different and may depend on the presence of EBV.[96]

Although an extensive discussion of other parasites that might act as cofactors in Burkitt lymphomagenesis is beyond the scope of this review, it is worth making a brief comment on another parasite, the protozoon *Plasmodium falciparum* that causes malaria, as there is a striking overlap in the geographic incidence of eBL and this disease. Even though not much is known about the mechanisms that the parasite uses to contribute to tumor formation, recent literature shows that *P. falciparum* infection drives EBV-infected cells through GC, and it is capable of deregulating the expression of the *AICDA* gene (also referred to as *AID*), which would lead to chromosomal translocations, as we mentioned earlier.[97–99] Translocations would mainly occur in EBV-infected cells within the GC that more likely would tolerate it. This observation reinforces the speculation that other cofactors are required for the occurrence of BL in endemic areas and that these parasites play an active role in Burkitt lymphomagenesis.

miRNAs as prognostic indicators of BL

Given the clear involvement of miRNA deregulation in the pathogenesis of BL, their diagnostic and prognostic value has been evaluated. A recent study reports that the identification of three circulating miRNAs (miRNA-21, miRNA-23a and miRNA-125b) in the plasma of BL patients may be used as a diagnostic indicator and could be related to clinicopathologic parameters.[100] Increased expression was observed for miR-21 and miR-23, and it was also correlated with some clinicopathologic parameters (tumor staging, increased white blood cells, increased serum lactate dehydrogenase level, CD10 expression and size of the tumor >6 cm).[100] Very interestingly, the expression level of these miRNAs decreased significantly following chemotherapy, suggesting that these miRNAs could be used to monitor therapy efficacy.[100] Also, an inverse correlation between the level of these miRNAs and patients' outcome was established, indicating these miRNAs act as prognostic indicators as well.[100] Very recently, the expression of another miRNA (hsa-miR-10a-5p) has been linked to the prognosis of BL patients, with this being downregulated in non-survivors.[101] Interestingly, genes targeted by this miRNA are involved in control of apoptosis and their overexpression could favor cell growth. Additionally, high expression of CD59 as a result of hsa-miR-10a-5p imbalance may determine reduced sensitivity to chemo- and immunotherapy and explain treatment failure and reduced overall survival in BL.[101]

miRNAs as potential targets in novel treatments for BL

BL is classically treated by a combination of chemotherapy and immunotherapy.[102] Nevertheless, due to the aggressiveness of this tumor, it is imperative to explore more effective therapeutic alternatives. One such possibility would be to

target miRNAs to either suppress or induce the expression of target genes, which might be relevant for a better prognostic outcome. Recent literature is providing useful information about new drugs or possible new therapeutic targets, including miRNAs. An obvious target for BL treatment would be *MYC* and its related network. A recent study reports that the use of INZ(c), a second generation of Inauhzin, is able to suppress Myc expression and it results in inhibition of cell growth in lymphoma cells.[103] Suppression of *MYC* is achieved through the miRNA pathway, as the expression of *MYC*-targeting miRNAs, such as miR-24 and miR-34a, is induced upon treatment to reduce Myc levels.[103] Noteworthy, this small molecule does not have considerable side effects and could be used in combination with doxorubicin to reduce Myc expression, allowing the administration of a lower dose of doxorubicin with consequent reduction of side effects.[103] However, due to the pleiotropic activities regulated by *MYC*, it is very difficult to design therapeutic approaches to inhibit its expression in human tumors without interfering with its physiological functions, and other potential targets should be explored.

Treatment with the combination of histone deacetylase inhibitor and chemotherapy results in induction of apoptosis in BL cells through the proapoptotic BCL2-related family member Bim protein.[104] A recent study of the same group describes that the use of combination of histone deacetylase inhibitor and chemotherapy could prevent cell growth in BL by regulating PI3K/Akt, suggesting that other targets, such as the PI3K/Akt signaling network, in addition to *MYC* should be further explored.[105] The combination of demethylating agents and chemotherapy could be used to revert the expression of aberrantly silenced genes and miRNAs, such as p16 and miR-101, miR-143 and miR-145, in BL tumor models.[105] Of these, miR-145 directly targets *MYC* and is expressed through the PI3K pathway, which is deregulated in BL.[106] Re-expression of miR-145 by this combinatorial approach may, therefore, result in reduction of *MYC* expression levels.[105]

Regulation of cell proliferation and induction of a more differentiated phenotype could be another possible approach as BL is the fastest growing tumor. It has been recently reported that the re-expression of miR-150 could be used as a possible promising therapeutic target because of its capability of reducing cell proliferation by targeting B-Myb.[107,108] In addition, this would result in the acquisition of a more differentiated phenotype as BL results from an impairment during differentiation toward plasma cells.[109,110]

Conclusion

MYC overexpression is the hallmark of BL and it can be consequent to several pathogenetic mechanisms. Recent literature highlights the key role of miRNAs in the pathogenesis of BL that can imbalance Myc and its associated pathways. Detection of miRNA expression can be used for diagnostic and therapeutic purposes. Re-expression of endogenous miRNAs through the administration of demethylating drugs to revert their silencing, or ectopic introduction of exogenous small RNAs that target deregulated genes could represent an exciting alternative to current therapies to improve the overall survival and reduce the side effects in BL patients. However, despite encouraging results, there is still much to uncover before such innovative therapeutic approaches may enter daily practice, and research in the field should be pursued to better clarify their role in the pathogenesis of BL.

Disclosure

The authors report no conflicts of interest in this work.

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