In: Primary Central Nervous System Lymphoma (PCNSL) ISBN: 978-1-63485-322-4
Editor: Ryuya Yamanaka

Chapter 8

MICRORNAS REGULATION AND PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

Tomohiko Kanayama[1,*], Azusa Hayano[2] and Ryuya Yamanaka[1,2]
[1]Laboratory of Molecular Target Therapy for Cancer,
Graduate School of Medical Science,
Kyoto Prefectural University of Medicine, Kyoto, Japan
[2]Department of Medical Science, Graduate School for Health Care Science,
Kyoto Prefectural University of Medicine, Kyoto, Japan

ABSTRACT

MicroRNAs (miRNAs) are non-coding regulatory RNAs, consisting of 19–25 nucleotides. Small miRNAs act through RNA interference mechanisms to regulate various cellular processes, including cell proliferation, differentiation, angiogenesis, and apoptosis in various organs and tissues, and require two RNase III complexes for activation. Unlike exogenous small interfering RNAs (siRNAs), miRNA can inhibit the translation of many mRNA sequences through the RNA-induced silencing complex, when its sequence is imperfectly matched with the target mRNA sequence. The general miRNA biogenesis pathway has been well established over a decade of intensive research in during which increases and decreases in miRNA expression have been reported in various organ samples from patients with a range of diseases, including cancer. Regarding cancer, in an early study, Calin and colleagues reported a link between miRNA dysregulation and tumor malignancy in chronic lymphocytic leukemia. Recently, Fischer and colleagues reported differential miRNA expression in nodal and non-nodal lymphomas, such as primary central nervous system lymphoma (PCNSL).

Among various miRNAs, miR-21, the most abundant miRNA in patients with PCNSL, has been well characterized as an oncogenic miRNA. In addition, several groups reported miR-21 as a strong candidate biomarker in the serum and cerebrospinal fluid (CSF) from patients with PCNSL. This chapter focuses on miRNA biology in cancer and the studies about miRNA expression and regulation in PCNSL.

* Corresponding Author address: Laboratory of Molecular Target Therapy for Cancer, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan. Email: tomokana@koto.kpu-m.ac.jp.

Keywords: microRNA, PCNSL, biomarker

INTRODUCTION

Primary central nervous system lymphoma (PCNSL) is a rare subtype of extra-nodal non-Hodgkin's lymphoma (NHL). It most commonly occurs in the brain, and though it accounts for only 2%–3% of NHL cases, its incidence has been increasing. More than 90% of PCNSLs are diffuse large B-cell lymphomas (DLBCLs). PCNSL is usually an aggressive lymphoma, and is characterized by a poorer prognosis than other systemic lymphomas with similar histological features [1].

The central nervous system (CNS) normally lacks a lymphatic system, and therefore, the mechanisms underlying DLBCL development in the CNS remain unclear. Recent studies have revealed the dysregulation of microRNA (miRNA) expression in cancer cells [2-5]. The mature miRNAs, which are 19–25 nucleotides long, non-coding RNAs, can regulate gene expression by binding to partially complementary target sites in the 3′ untranslated regions (UTRs) of target mRNAs, resulting in the degradation of target mRNAs or translational repression of the encoded proteins via several mechanisms [6-8].

Some specific miRNAs downregulate the expression of tumor suppressor genes such as *P53* and *PTEN*, thus promoting tumorigenesis (onco-miRNA [9-13]), while others downregulate the expression of oncogenes such as *MYC* and *BCL2*, thus exerting tumor suppressor effects in cancer cells (tumor suppressor-miRNA [9, 14-17]). To understand the cancer-specific miRNA signature, the characteristics of miRNA expression have been compared between cancer cells and other diseased cells or normal control cells by using overall miRNA expression analyses such as microarray analysis and miRNA sequencing analysis [18-20]. In the span of a decade, numerous miRNAs have been identified through the development of several detection techniques. From miRBase (http://www.mirbase.org/ release 21, updated June 2014) information, over 2500 human mature miRNAs have been registered so far. Many of these have not been characterized completely and their functions in human organs, including the brain, remain unclear. Several studies have focused on the well-known and well-characterized miRNAs and their roles in disease and cell development, their target genes, and expression patterns [21]. In this chapter, recent studies on miRNA expression and function in lymphomas, mainly DLBCL and PCNSL, are discussed. To develop a novel diagnostic biomarker for PCNSL, recent studies focused on miRNA expression analysis in cerebrospinal fluid (CSF) and serum are also discussed.

1. REGULATION OF miRNA EXPRESSION IN CANCER

1.1. miRNA Biogenesis in Cancer

miRNAs are generally transcribed by RNA polymerase II (RNA pol II) as long primary transcripts from various genomic regions, including genes (exons and introns) and other non-coding regions (Figure 1a) [6, 22]. Primary miRNAs (pri-miRNAs) (>1 kbp in length) have characteristic hairpin structures and are converted to precursor miRNAs (pre-miRNAs; 70–

100 bp) by Drosha/DiGeorge syndrome critical region gene 8 (DGCR8) RNase III complex (Figure 1 b) [6, 22]. The pre-miRNAs are exported from the nucleus into the cytoplasm via an exportin 5-mediated mechanism (Figure 1c) [6, 22], where the action of another RNase III, Dicer, generates short mature miRNAs (19–25 bp) (Figure 1d). The mature miRNAs are incorporated into a large protein complex termed the RNA-induced silencing complex (RISC) [6, 22]. The mature miRNA duplex is recognized by the Argonaute (Ago) protein-containing RISC-loading complex (RLC) and is unwound into two single-stranded RNAs, the guide strand and the passenger strand (Figure 1e). The passenger strand (designated as miRNA* in Figure 1) is degraded, and the guide strand RNA is incorporated into the RISC. The binding of the miRNA guide strand with target mRNAs occurs via approximately 7 nucleotide-long stretches, the so-called "seed sequence," present in the 5′ region of the miRNA. The mature single-stranded miRNA regulates the expression of target genes via imperfect binding with the 3′ UTR of target mRNAs through the Ago-containing RISC (Figure 1f) [6, 22]. Besides this canonical mechanism of miRNA-mediated gene regulation, non-canonical pathways such as Drosha/ DGCR8-independent miRNA biogenesis have been reported [7]. RISC-mediated target mRNA silencing is promoted by mRNA degradation through the recruitment of decapping and deadenylation enzymes to the miRNA-bound mRNA, or is performed by translation initiation and elongation inhibition by inhibition of ribosomal subunit recruitment into the translational 80S ribosomal complex [7, 22].

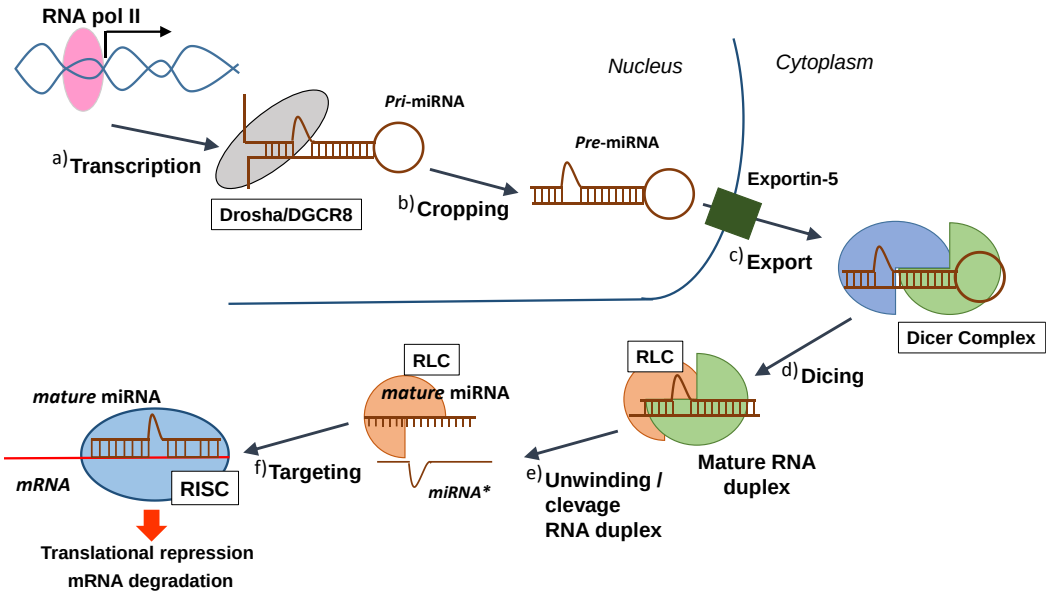

Figure 1. General mechanism of miRNA biogenesis.

1.2. Genome Amplification and Deletion

miRNA transcripts are derived from various genomic regions (e.g., introns and exons of genes, non-coding regions), and most miRNAs are transcribed by RNA pol II. Therefore, miRNA expression is affected by chromosomal alterations such as loss of heterozygosity (LOH) and amplifications such as translocations and single-nucleotide polymorphisms [23,

24]. Calin et al. [25] first reported that LOH in 13q14, where miR-15a/miR-16-1 is located, constitutes the most frequent chromosomal deletion in chronic lymphocytic leukemia (CLL). The most common chromosomal abnormalities in CLL detectable by cytogenetics include deletions in 13q (55%), 11q (18%), and 17p (8%), and trisomy 12 (12%–16%) [23, 26]. Further investigation revealed that miR-15a/miR-16-1 and miR-34 families (miR-34b/c located in 11q23) are transcriptionally inducible by p53 (located in 17p13). Fabbri et al. showed that miR-15a/miR-16-1 binds to the 3′ UTR of *P53* mRNA to suppress p53 activity; on the other hand, they also reported that the miR-34 family binds to the coding region of *ZAP70* to suppress ZAP70 [26], and the high expression of ZAP70 serves as a poor prognostic factor of CLL [27]. In this way, 11q/13q/17p linkage-derived miRNA/p53 feedback circuitry is associated with the pathogenesis and prognosis of CLL. These findings may explain the model for the indolent form of CLL involving 13q deletions, in which increased p53 levels are associated with transactivation of miR-34b/c and reduced levels of ZAP70 [26].

Tagawa et al. reported genome amplification at 13q31-32 in malignant DLBLC [28, 29]. Six miRNA genes (miR-17, miR-18, miR-19a/b, miR-20a, and miR-92-1) are clustered within the non-protein-coding gene C13orf25 (13q31). With a 13q31 gain/amplification, the higher expressions of these miRNAs are comparable with their expressions without 13q31 amplification [30].

Calin et al. pointed out that many miRNA genes are located in cancer-associated genomic regions or in fragile sites in which sister chromatid exchange, translocation, deletion, and amplification is frequent [31]. These regions include the chromosomal regions mentioned above.

1.3. miRNA Clusters and miRNA Transcription

Several miRNA clusters, such as C13orf25 (miR-17-92 cluster) in 13q31, and their association with diseases including cancer, have been the subjects of extensive research [30-36]. Previous studies have found that a substantial fraction of miRNAs is located in polycistronic transcripts, implying a common phenomenon of miRNA clustering. Clustered miRNAs are generally similar in sequence (e.g., miR-222/221, miR-29a/29b, miR-15a/16-1 clusters), but can sometimes differ (e.g., miR-17-92, miR-106b/93/25, miR-106a-363 clusters) [37-40].

The expression of the miR-17-92 cluster—a well-studied miRNA cluster—is regulated by the transcription factor (TF) Myc [12, 40]. In patients with CLL, the expression of some miRNAs in this cluster is different from that of others, depending on *P53* expression [41]. For example, a loss of p53 results in high expression of miR-19/miR-92a (onco-miRNA) and low expression of miR-17/miR-20 (tumor suppressor-miRNA). The normal expression of *P53* leads to a high miR-17/miR-20 expression. The relationship between p53 and imbalanced miR-17-92 expression suggests that increased expression of oncogenic miR-17-92 members and decreased expression of tumor suppressive members probably accelerates disease progression [41]. Considering that miR-18 expression remains unchanged in patients with CLL compared with that in normal controls, another speculation is that miR-18 may be subjected to modification during its maturation process to induce specific miRNA degradation [29, 42].

On one hand, while Myc regulates the transcription of several miRNA clusters, tumor-suppressor miRNAs directly targeting *MYC* have also been well-studied [40, 43-46]. Interestingly, the tumor suppressor gene *P53* regulates miRNA expression, and onco-miRNAs that directly target *P53* have also been reported [47]. In this manner, the TF-miRNA circuit can influence diverse oncogenesis-related processes, such as cell cycle regulation, apoptosis, metabolism, angiogenesis, epithelial to mesenchymal transition, and metastasis. Therefore, miRNA dysregulation has been suggested as an important mechanism for the aberrant expression of a list of oncogenes and/or tumor suppressor genes that are not affected directly by genetic mutations or transcriptional regulation in tumorigenesis.

1.4. Epigenetic Regulation of miRNA Expression

Aberrant epigenetic changes are a well-known feature of cancer cells, such as DNA hypermethylation of tumor suppressor genes involving DNA methyltransferases (DNMT1 or DNMT3A and DNMT3B), extensive genomic DNA hypomethylation, and alteration of histone modification patterns such as acetylation, methylation, and phosphorylation [48-50]. Similar to protein coding genes, miRNA genes are also subject to epigenetic changes in cancer. A large proportion of miRNA loci are associated with CpG islands, giving a strong basis to their regulation by DNA methylation. Most studies have used treatment with chromatin remodeling drugs (e.g., DNMT inhibitors such as 5-aza-2′-deoxycytidine) to reveal epigenetically silenced miRNAs. Global DNA methylation analysis further revealed an extensive influence of DNA hypermethylation and hypomethylation on the regulation of miRNA expression. Most studies on the epigenetic silencing of miRNA genes in cancer have focused on CpG island hypermethylation [48-50]. By contrast, Dudziec et al. demonstrated that the methylation of CpG island shores, which are regions located within 2 kb of CpG islands, strongly affects gene expression and is frequently associated with the downregulation of miRNA expression in bladder cancer [51].

In a study by Baer et al. on the epigenetic regulation of miRNAs in CLL, promoters for 781 miRNAs were identified by upstream H3K4 me3 enrichment by using a panel of leukemic cell lines, primary CLL cells, and healthy B cells. Over 100 miRNAs were found to be the targets of recurrent aberrant methylation in CLL cells. Hypomethylation accounted for more than 60% of differential methylation [50]. Moreover, while DNA hypermethylation occurred preferentially in CpG islands, hypomethylation was almost exclusively found adjacent to the CpG islands or in the CpG-free promoters [50]. The patterns of hypomethylation are in concordance with the observations of extensive genome-wide DNA hypomethylation in CLL [52]. Inverse correlations between DNA methylation and the expression of miRNAs, such as mir-129-2, mir-551b, and mir-708 (hypermethylated miRNAs) and mir-21, mir-34a, mir-135a, mir-155, mir-574, mir-664, mir-1204, and the cluster mir-29a/29b-1 (hypomethylated miRNAs) were found to be DNA methylation-dependent in CLL [50].

Other than DNA methylation, histone acetylation represents another dysregulated epigenetic phenomenon in cancer. A decrease in the level of acetylated histones can downregulate the expression of anti-oncogenic miRNAs, as evidenced by studies utilizing histone deacetylase inhibitors (e.g., 4-phenylbutyric acid) [53].

Meanwhile, DNA or histone modification enzymes are also targets of dysregulated miRNAs in cancer. Fabbri et al. first reported that the miR-29 family, whose expression is downregulated in cancer, can target DNMT3 [54], and that the ectopic expression of miR-29 family in lung cancer could restore methylation at loci containing tumor suppressor genes (e.g., those encoding WWOX and FHIT), which are aberrantly methylated in lung cancer [54]. A study by Fabbri et al. showed that DNMT3A is a prognostic factor in lung cancer, and the overexpression of miR-29 suppressed tumorigenicity [54]. Moreover, further studies revealed that miR-29 downregulates the expression of oncogenes such as *TCL1*, *MCL1*, and *BCL2L2* and indirectly activates the p53 pathway through p85a (PI3K subunit) targeting [55]. It also became apparent that in cancer, DNMTs are regulated by several miRNAs (e.g., miR-148a, miR-143) [56-58].

Some miRNAs (e.g., miR-101, miR-26a) affect chromatin modification by targeting EZH2, another important epigenetic factor. EZH2 is a member of the polycomb group of proteins, which are key regulators that silence numerous developmental genes. EZH2 is the catalytic subunit of polycomb repressive complex 2, and trimethylates H3K27 [58].

1.5. Alterations in miRNA Processing

The expression of miRNA can also be modified as a result of defects in the miRNA biogenesis pathway (Figure 1). Dysregulation of the enzymes and cofactors involved in these post-transcriptional pathways can affect the levels of mature miRNAs and have important biological effects. In the canonical pathway, two RNase III enzyme complexes—the Drosha/DGCR8 complex and the Dicer complex—are important for the complete maturation of miRNA [59]. Analysis of mature and pri-miRNA expression in tumor and normal samples revealed that the correlation between pri-miRNA and mature miRNA was disrupted in tumor samples [59]. Available evidence suggests global alteration in miRNA processing in cancer. Knockdown of Drosha, DGCR8, or Dicer increased tumorigenicity in mouse lung adenocarcinoma [60, 61], while knockout of p68 or p72, other Drosha/DGCR8 microprocessor complex components, in mice resulted in embryonic lethality. Studies focused on proteins interacting with p68 have revealed that several TFs, such as p53, Smads, and estrogen receptor-α, positively regulate microprocessor activity [62-64]. Similarly, hippo tumor suppressor signaling related Yes-associated protein 1 negatively regulates microprocessor activity by interfering with the association of p72 with the microprocessor complex [65].

Recent studies have contributed toward elucidating the mechanism of expression of the key regulators involved in miRNA processing. The expression of the key components of miRNA biogenesis, including Dicer, is regulated by tumor-derived miRNAs such as the miR-103/107 family, miR-221/222 family, miR-29a, and miR-15a/16-1 [66-70]. In addition, the expression of Dicer is upregulated by miR-200c, which is associated with decreased migration and invasion in breast cancer [67].

2. MIRNA IN PCNSL

The molecular biology of PCNSL is not well understood. Two groups have published the "CNS" signatures of PCNSL, or the unique gene expression and pathway signatures, by comparing PCNSL with non-CNS DLBCL [71-74]. Considering the architecture of CNS lymphoma, there are at least two major growth patterns of PCNSL, which are characterized by low or high tumor cell density. High-density tumors that express activated STAT-6 are associated with a worse prognosis after treatment with standard methotrexate-based regimens than are tumors of low cell density, independent of STAT-6 activation status [71].

These observations coincided with previous studies in which PCNSL was found to share characteristics with systemic DLBCL with respect to the activated B cells (ABCs; e.g., immunoglobulin [Ig] M expression, lack of class switch recombination, NF-kB pathway activation) and the germinal center B cell (GCB) subtypes (high Ig gene mutation frequency with ongoing somatic hypermutation, BCL6 expression) [75-77]. This section will discuss the miRNA expression in DLBCL, including PCNSL.

In the later sections, the advancements in the investigation of biomarkers from patient samples brought about by a decade of developments in detection technologies are discussed.

2.1. miRNA Expression in PCNSL Tissue

In DLBCL, miRNAs are well characterized in terms of their roles in hematopoiesis and lymphomagenesis [78-81]. Roehle et al. reported altered miRNA expression in DLBCL compared with that in normal lymph nodes (upregulated: miR-155, miR-210, miR-17-5p, and miR-106a; downregulated: miR-150, miR-145, miR-328, miR-139, miR-99a, miR-10a, miR-95, miR-149, miR-320, miR-151, and let-7e) [82]. Malumbres et al. identified DLBCL subtype-specific miRNA signatures (i.e., specific to ABC-like-DLBCL or GCB-like-DLBCL) [83]. High expression of nine miRNAs in ABC-like-DLBCL has been identified (miR-146b, miR-146a, miR-21, miR-155, miR-500, miR-222, miR-363, miR-574-3p, and miR-574-5p).

With respect to the relationship between the expression of single miRNAs and survival in patients with DLBCL, Roehle et al. showed that a reduction in miR-127 levels significantly correlates with poor event-free survival (EFS) and overall survival (OS). MiR-127 was previously reported to be methylated in tumor cells, and the expression level was inversely proportional to the expression of BCL6, a known proto-oncogene [53]. Moreover, reduced expression of four miRNAs (miR-21, miR-23a, miR-27a, and miR-34a) was found to be associated with poor OS. Reduced miR-19 expression is correlated with poor EFS, whereas reduced expression of miR-195 and let-7g is associated with good EFS [82].

Malumbres et al. reported the correlation between the differential expression of miRNAs between GCB-like and ABC-like cell lines and the outcome of DLBCL patients treated with rituximab-CHOP (R-CHOP). The high expression of miR-222, characteristically expressed in ABC-like cell lines, was associated with inferior OS and progression-free survival (PFS). No correlations between the expression of miR-21 or miR-155 and OS or PFS were observed [83]. Meanwhile, Iqbal et al. reported that high expression of three miRNAs (miR-16, miR-155, and miR-363) was significantly associated with R-CHOP therapy failure in DLBCL, whereas high expression of miR-24 was associated with a favorable treatment response.

According to this report, the low expression of three genes—KLHL5, PSIP1, and CHD9—regulated by miR-155 had a significant correlation with poor EFS in ABC-like DLBCL, but not in GCB-DLBCL [84].

Fischer et al. reported that the expression of 13 miRNAs (miR-9, miR-20b, miR-155, miR-340, miR-17-5p, miR-148a, miR-30b, miR-27b, miR-26b, miR-146b, miR-20a, miR-30c, and let-7g) was significantly higher in PCNSL than in nodal DLBCL [84]. Meanwhile, the expression of five miRNAs (miR-199a, miR-214, miR-432, miR-193b, and miR-145) was reduced in PCNSL compared with that in nodal DLBCL. Of the 13 miRNAs whose expression was upregulated in PCNSL, miR-9, miR-20b, miR-155, miR-340, miR-17-5p, and miR-148a showed the highest expression [85].

Fischer's study also showed that miR-155 and miR-92, members of the miR-17-92 cluster, were highly expressed in both PCNSL and nodal DLBCL. Other than miR-92, other members of the mir-17-92 family (miR-17-5p, miR-20a, and miR-20b) were also upregulated in PCNSL. The expression of miR-17-92 family members is upregulated via the Myc pathway, thus accelerating Myc-induced lymphoma development [85]. In PCNSL, downregulation of miR-145 expression, which directly suppress the expression of Myc, also suggests Myc pathway activation.

Moreover, upregulation of the expression of miR-9 and miR-30 families in PCNSL might cause a Myc activation-mediated block in B cell terminal differentiation through the inhibition of PRDM-1-mediated Myc repression. In GCBs, miR-9 and miR-30 are overexpressed, leading to the downregulation of PRDM-1, a regulator of plasma cell differentiation [86, 87]. These results could shed light on the stronger Myc expression observed in PCNSL than in nodal DLBCL [73].

In addition, miR-17-92 [88, 89] and miR-155 [90-92] have been reported to exhibit oncogenic properties in lymphoma [21]. Notably, miR-155 upregulation has been reported in response to immune stimuli such as tumor necrosis factor-α, and this miRNA has been demonstrated to attenuate TGF-β-mediated activation by retinoblastoma protein [92].

Here, miR-21 overexpression has been observed in many types of cancers including lymphomas [8]. The role of miR-21 in cancer has been characterized by using *in vitro* and *in vivo* models [10-11]. These studies have identified miR-21 as an onco-miRNA to which some tumors including lymphoma are "addicted" [11]. MiR-21 expression is significantly elevated in both DLBCL and PCNSL [93]. Interestingly, miR-21 higher expression is observed in activated B cells response to IL-4, helper T-cell produced cytokine [94]. These results suggested miR-21 may help maintain B cell hyperactivation in lymphomagenesis [5]. As mentioned above, miR-21 is robustly expressed in DLBCLs, miR-21 has been regarded as a candidate of most potent biomarker from biomaterials (e.g., serum, CSF) in patient with DLBCLs.

Robertus et al. reported the expression of 15 miRNAs by using qRT-PCR (miR-21, miR-19, miR-15b, miR-92a, miR-20a, miR-15a, miR-19a, miR-17-5p, miR-181a, miR-155, miR-18a, miR-221, miR-17-3p, miR-127, and miR-16). In DLBCL, miR-21 and miR-19b are highly expressed; in particular, miR-21 has a 100-fold higher expression level and miR-19b has a 10-fold higher expression level compared with other miRNAs [92]. Robertus' study especially showed that miR-17-5p and miR-127 are candidates for markers that can distinguish between PCNSL and other extra-nodal testicular DLBCLs [93].

2.2. miRNA as a Biomarker in Patients with PCNSL

Identification of the origin of a disease is critical to successful treatment. Therefore, if miRNAs could distinguish tumor origins, subtypes, oncogenic mutations, and cancer predispositions, and regulate the most important cellular processes, it is logical to hypothesize that these elements can help predict cancer prognosis and/or responses to specific therapies. Particularly, the development of non-invasive techniques is crucial for facilitating diagnostic analysis. For this, blood samples from patients are most convenient for the detection of cancer-specific biomarkers. CSF is also suitable for detection, follow-up, and prognostication in CNS diseases such as PCNSL and brain cancer [95-98].

The use of non-coding RNAs (miRNAs in particular) has gained significant attention since the discovery that these RNA species could be detected both extra-and intracellularly in peripheral tissues and as free or exosomal RNAs in culture medium from tumor cells. Regarding the tumor-derived RNAs in culture medium, these RNAs have also been detected as circulating free or exosomal RNAs in blood and CSF [98]. The increasing use of powerful detection techniques such as massive sequencing has significantly boosted the search for minimally invasive disease indicators [96]. This technology is rapidly evolving and promises great advances in the field of biomarker discovery, particularly in the area of nervous system pathology. The CNS is the least accessible of all tissues; therefore, advances in this field will be of great benefit. Similar to detection technologies, several advanced methods for extraction and preparation of RNAs from tissue samples have been developed.

These strategies accelerate the exploration of miRNA profiles in the serum and CSF of patients with various cancers [94, 99-103].

As mentioned above, in various cancers, increased miR-21 expression is an indicator of poor outcome. However, in DLBCL, decreased miR-21 expression indicates poor prognosis. A recent study showed higher expression of miR-21, miR-155, and miR-210 in the serum of patients with DLBLC than in normal controls [104]. Thus, high miR-21 levels in serum are associated with a more favorable clinical outcome in DLBCL [104]. Fang et al. also reported a higher expression of miR-21 in the serum of patients with DLBCL. Elevated levels of miR-155, miR-15, miR-16, and miR-29c and low levels of miR-34a have been detected in the serum of patients with DLBCL [105].

Baraniskin et al. reported the expression of six miRNAs (miR-21, miR-19b, miR-92a, miR-15b, miR-106b, and miR-204) and control miRNA (miR-24) in the CSF taken from patients with PCNSL and healthy controls. A higher expression of three candidate miRNAs (miR-21, miR-19b, and miR-92) was observed in the CSF of patients with PCNSL than in healthy controls. The expression of these three miRNAs in the CSF also distinguishes PCNSL from other diseases derived from the CNS. Hence, these miRNAs are potential candidates for biomarkers [106]. Baraniskin et al. also reported miRNA expression in the CSF of patients diagnosed with PCNSL [107]. The expression of miR-21, miR-19b, and miR-92 was monitored in patients with different clinical statuses. The difference in patient status (e.g., complete remission, recurrence after initial partial remission, disease recurrence following complete remission) was reflected in the differences in miRNA expression. In the case of complete remission, these three miRNAs were undetectable in the CSF [107]. Another study by Baraniskin showed that the expression of miR-15b and miR-21 in CSF could be used to distinguish between PCNSL and glioma [108]. Moreover, in other tumors, such as classical Hodgkin lymphoma, serum miRNA levels were observed to change after therapy [99, 109].

Mao et al. reported conflicting data about the correlation between serum and CSF miR-21 levels and prognosis in patients with PCNSL [110]. These conflicting results need to be confirmed through additional analyses by using different datasets.

Roth et al. recently reported an analysis of serum miRNA expression patterns in PCNSL patients by using next-generation sequencing and qRT-PCR. In that study, distinctions between PCNSL patients with long-term and short-term survival were validated by using miRNA. Twelve miRNAs (miR-151-a, miR-151-b, miR-106a-5p, miR-219-5p, miR-17-5p, miR-6130, miR-183-3p, miR-30d-5p, miR-181c-3p, miR-96-5p, miR-194-5p, and miR-503-5p) were found to differ significantly between the two groups [111].

From these results, despite available data regarding miRNA expression in rare CNS disease subtypes, datasets specific to PCNSL remain few in number, and the characteristics of PCNSL remain unclear. However, a large-data, comprehensive analysis on DLBCL was published in 2015 [19]. Similar future research concerning PCNSL will facilitate the understanding of characteristics associated with an improved prognosis.

In a previous report, microarray profiling in combination with real-time RT-PCR and locked nucleic acid-based in situ hybridization, uncovered 44 miRNAs expressed in the adult mouse brain network. Interestingly, >3-fold enrichment of this miRNA profile was detected in the spinal cord, cerebellum, medulla oblongata, pons, hypothalamus, hippocampus, neocortex, olfactory bulb, eye, and pituitary gland [112]. Various expression patterns in different areas of the brain indicate the complexity of brain development. Such studies might clarify the etiologies of brain diseases through the analysis of miRNA expression in the human brain.

CONCLUSION

The past decade yielded an explosion of research focused on small non-coding RNAs, such as exogenous siRNAs and endogenous miRNAs. Progressive improvements in analytical techniques have revealed patterns of miRNA dysregulation in various cancers.

Currently, researchers continue to encounter difficulties in the sampling and preparation of materials suitable for diagnostic procedures. To improve the detection of small molecules such as miRNAs in blood and CSF samples, we must distinguish between the normal tissue microenvironment and disease-derived molecules. After another decade of research and the use of advanced miRNA-RNA network tools, we expect that the functions of most of the poorly-understood miRNAs will be elucidated.

REFERENCES

[1] Ferreri AJ, Marturano E. (2012) Primary CNS lymphoma. *Best Pract Res Clin Haematol.*, 25, 119-30.

[2] Lawrie CH. (2013) MicroRNAs and lymphomagenesis: a functional review. *Br J Haematol.*, 160, 571-81.

[3] Tagawa H, Ikeda S, Sawada K. (2013) Role of microRNA in the pathogenesis of malignant lymphoma. *Cancer Sci.*, 104, 801-9.

[4] Lawrie CH, Saunders NJ, Soneji S, Palazzo S, Dunlop HM, Cooper CD, Brown PJ, Troussard X, Mossafa H, Enver T, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS. (2008) MicroRNA expression in lymphocyte development and malignancy. *Leukemia,* 22, 1440-6.

[5] Jardin F, Figeac M. (2013) MicroRNAs in lymphoma, from diagnosis to targeted therapy. *Curr Opin Oncol*., 25, 480-6.

[6] Davis-Dusenbery BN, Hata A. (2010) MicroRNA in Cancer: The Involvement of Aberrant MicroRNA Biogenesis Regulatory Pathways. *Genes Cancer*., 1, 1100-14.

[7] Li Z, Rana TM. (2014) Therapeutic targeting of microRNAs: current status and future challenges. *Nat Rev Drug Discov*., 13, 622-38.

[8] Iorio MV, Croce CM. (2012) MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med*., 4, 143-59.

[9] Zhang B, Pan X, Cobb GP, Anderson TA. (2007) microRNAs as oncogenes and tumor suppressors. *Dev Biol*., 302, 1-12.

[10] Yang CH, Pfeffer SR, Sims M, Yue J, Wang Y, Linga VG, Paulus E, Davidoff AM, Pfeffer LM. (2015) The oncogenic microRNA-21 inhibits the tumor suppressive activity of FBXO11 to promote tumorigenesis. *J Biol Chem*., 290, 6037-46.

[11] Medina PP, Nolde M, Slack FJ. (2010) OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. *Nature,* 467, 86-90.

[12] Dal Bo M, Bomben R, Hernández L, Gattei V. (2015) The MYC/miR-17-92 axis in lymphoproliferative disorders: A common pathway with therapeutic potential. *Oncotarget,* 6, 19381-92.

[13] Babar IA, Cheng CJ, Booth CJ, Liang X, Weidhaas JB, Saltzman WM, Slack FJ. (2012) Nanoparticle-based therapy in an in vivo microRNA-155 (miR-155)-dependent mouse model of lymphoma. *Proc Natl Acad Sci U S A.,* 109, E1695-704.

[14] Saito Y, Nakaoka T, Saito H. (2015) microRNA-34a as a Therapeutic Agent against Human Cancer. *J Clin Med*., 4, 1951-9.

[15] Yamakuchi M, Ferlito M, Lowenstein CJ. (2008) miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci U S A.,* 105, 13421-6.

[16] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA, Hannon GJ. (2007) A microRNA component of the p53 tumour suppressor network. *Nature,* 447, 1130-4.

[17] Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M, Taccioli C, Zanesi N, Garzon R, Aqeilan RI, Alder H, Volinia S, Rassenti L, Liu X, Liu CG, Kipps TJ, Negrini M, Croce CM. (2008) MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc Natl Acad Sci U S A., 105,* 5166-71.

[18] Gattolliat CH, Uguen A, Pesson M, Trillet K, Simon B, Doucet L, Robaszkiewicz M, Corcos L. (2015) MicroRNA and targeted mRNA expression profiling analysis in human colorectal adenomas and adenocarcinomas. *Eur J Cancer*, 51, 409-20.

[19] Lim EL, Trinh DL, Scott DW, Chu A, Krzywinski M, Zhao Y, Robertson AG, Mungall AJ, Schein J, Boyle M, Mottok A, Ennishi D, Johnson NA, Steidl C, Connors JM, Morin RD, Gascoyne RD, Marra MA. (2015) Comprehensive miRNA sequence analysis reveals survival differences in diffuse large B-cell lymphoma patients. *Genome Biol.,* 16:18.

[20] Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Ménard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S,

Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. (2005) MicroRNA gene expression deregulation in human breast cancer. *Cancer Res*., 65, 7065-70.

[21] Calin GA, Croce CM. (2006) MicroRNA signatures in human cancers. *Nat Rev Cancer*., 6, 857-66.

[22] Jansson MD, Lund AH. (2012) MicroRNA and cancer. *Mol Oncol*., 6, 590-610.

[23] Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L, Döhner K, Bentz M, Lichter P. (2000) Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*., 343, 1910-6.

[24] Gong J, Tong Y, Zhang HM, Wang K, Hu T, Shan G, Sun J, Guo AY. (2012) Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. *Hum Mutat*., 33, 254-63.

[25] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. (2002) Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A.*, 99, 15524-9.

[26] Fabbri M, Bottoni A, Shimizu M, Spizzo R, Nicoloso MS, Rossi S, Barbarotto E, Cimmino A, Adair B, Wojcik SE, Valeri N, Calore F, Sampath D, Fanini F, Vannini I, Musuraca G, Dell'Aquila M, Alder H, Davuluri RV, Rassenti LZ, Negrini M, Nakamura T, Amadori D, Kay NE, Rai KR, Keating MJ, Kipps TJ, Calin GA, Croce CM. (2011) Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. *JAMA.*, 305, 59-67.

[27] Dürig J, Nückel H, Cremer M, Führer A, Halfmeyer K, Fandrey J, Möröy T, Klein-Hitpass L, Dührsen U. (2003) ZAP-70 expression is a prognostic factor in chronic lymphocytic leukemia. *Leukemia*, 17, 2426-34.

[28] Tagawa H, Tsuzuki S, Suzuki R, Karnan S, Ota A, Kameoka Y, Suguro M, Matsuo K, Yamaguchi M, Okamoto M, Morishima Y, Nakamura S, Seto M. (2004) Genome-wide array-based comparative genomic hybridization of diffuse large B-cell lymphoma: comparison between CD5-positive and CD5-negative cases. *Cancer Res.*, 64, 5948-55.

[29] Tagawa H, Seto M. (2005) A microRNA cluster as a target of genomic amplification in malignant lymphoma. *Leukemia*, 19, 2013-6.

[30] Mogilyansky E, Rigoutsos I. (2013) The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ.*, 20, 1603-14.

[31] Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A.*, 101, 2999-3004.

[32] Navarro A, Gaya A, Martinez A, Urbano-Ispizua A, Pons A, Balagué O, Gel B, Abrisqueta P, Lopez-Guillermo A, Artells R, Montserrat E, Monzo M. (2008) MicroRNA expression profiling in classic Hodgkin lymphoma. *Blood*, 111, 2825-32.

[33] Li C, Kim SW, Rai D, Bolla AR, Adhvaryu S, Kinney MC, Robetorye RS, Aguiar RC. (2009) Copy number abnormalities, MYC activity, and the genetic fingerprint of normal B cells mechanistically define the microRNA profile of diffuse large B-cell lymphoma. *Blood*, 113, 6681-90.

[34] Troppan K, Wenzl K, Deutsch A, Ling H, Neumeister P, Pichler M. (2014) MicroRNAs in diffuse large B-cell lymphoma: implications for pathogenesis, diagnosis, prognosis and therapy. *Anticancer Res*., 34, 557-64.

[35] Lu TX, Young KH, Xu W, Li JY. (2016) TP53 dysfunction in diffuse large B-cell lymphoma. *Crit Rev Oncol Hematol*., 97, 47–55.

[36] Yu J, Wang F, Yang GH, Wang FL, Ma YN, Du ZW, Zhang JW. (2006) Human microRNA clusters: genomic organization and expression profile in leukemia cell lines. *Biochem Biophys Res Commun*., 349, 59-68.

[37] Wu HL, Heneidi S, Chuang TY, Diamond MP, Layman LC, Azziz R, Chen YH. (2014) The expression of the miR-25/93/106b family of micro-RNAs in the adipose tissue of women with polycystic ovary syndrome. *J Clin Endocrinol Metab*., 99, E2754-61.

[38] Dylla L, Jedlicka P. (2013) Growth-promoting role of the miR-106a~363 cluster in Ewing sarcoma. *PLoS One*, 8, e63032.

[39] Welten SM, Bastiaansen AJ, de Jong RC, de Vries MR, Peters EA, Boonstra MC, Sheikh SP, La Monica N, Kandimalla ER, Quax PH, Nossent AY. (2014) Inhibition of 14q32 MicroRNAs miR-329, miR-487b, miR-494, and miR-495 increases neovascularization and blood flow recovery after ischemia. *Circ Res*., 115, 696-708.

[40] Olive V, Jiang I, He L. (2010) mir-17-92, a cluster of miRNAs in the midst of the cancer network. *Int J Biochem Cell Biol*., 42, 1348-54.

[41] Li Y, Vecchiarelli-Federico LM, Li YJ, Egan SE, Spaner D, Hough MR, Ben-David Y. (2012) The miR-17-92 cluster expands multipotent hematopoietic progenitors whereas imbalanced expression of its individual oncogenic miRNAs promotes leukemia in mice. *Blood*, 119, 4486-98.

[42] Michlewski G, Guil S, Semple CA, Cáceres JF. (2008) Posttranscriptional regulation of miRNAs harboring conserved terminal loops. *Mol Cell*, 32, 383-93.

[43] Kumar P, Luo Y, Tudela C, Alexander JM, Mendelson CR. (2013) The c-Myc-regulated microRNA-17~92 (miR-17~92) and miR-106a~363 clusters target hCYP19A1 and hGCM1 to inhibit human trophoblast differentiation. *Mol Cell Biol*., 33, 1782-96.

[44] Zhao ZN, Bai JX, Zhou Q, Yan B, Qin WW, Jia LT, Meng YL, Jin BQ, Yao LB, Wang T, Yang AG. (2012) TSA suppresses miR-106b-93-25 cluster expression through downregulation of MYC and inhibits proliferation and induces apoptosis in human EMC. *PLoS One*, 7, e45133.

[45] Alencar AJ, Malumbres R, Kozloski GA, Advani R, Talreja N, Chinichian S, Briones J, Natkunam Y, Sehn LH, Gascoyne RD, Tibshirani R, Lossos IS. (2011) MicroRNAs are independent predictors of outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. *Clin Cancer Res*., 17, 4125-35.

[46] Jackstadt R, Hermeking H. (2015) MicroRNAs as regulators and mediators of c-MYC function. *Biochim Biophys Acta*., 1849, 544-53.

[47] Rokavec M, Li H, Jiang L, Hermeking H. (2014) The p53/microRNA connection in gastrointestinal cancer. *Clin Exp Gastroenterol*., 7, 395-413.

[48] Baer C, Claus R, Plass C. (2013) Genome-wide epigenetic regulation of miRNAs in cancer. *Cancer Res*., 73, 473-7.

[49] Lopez-Serra P, Esteller M. (2012) DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. *Oncogene*, 31, 1609-22.

[50] Baer C, Claus R, Frenzel LP, Zucknick M, Park YJ, Gu L, Weichenhan D, Fischer M, Pallasch CP, Herpel E, Rehli M, Byrd JC, Wendtner CM, Plass C. (2012) Extensive promoter DNA hypermethylation and hypomethylation is associated with aberrant microRNA expression in chronic lymphocytic leukemia. *Cancer Res*., 72, 3775-85.

[51] Dudziec E, Miah S, Choudhry HM, Owen HC, Blizard S, Glover M, Hamdy FC, Catto JW. (2011) Hypermethylation of CpG islands and shores around specific microRNAs and mirtrons is associated with the phenotype and presence of bladder cancer. *Clin Cancer Res*., 17, 1287-96.

[52] Kulis M, Heath S, Bibikova M, Queirós AC, Navarro A, Clot G, Martínez-Trillos A, Castellano G, Brun-Heath I, Pinyol M, Barberán-Soler S, Papasaikas P, Jares P, Beà S, Rico D, Ecker S, Rubio M, Royo R, Ho V, Klotzle B, Hernández L, Conde L, López-Guerra M, Colomer D, Villamor N, Aymerich M, Rozman M, Bayes M, Gut M, Gelpí JL, Orozco M, Fan JB, Quesada V, Puente XS, Pisano DG, Valencia A, López-Guillermo A, Gut I, López-Otín C, Campo E, Martín-Subero JI. (2012) Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia. *Nat Genet*., 44, 1236-42.

[53] Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, Jones PA. (2006) Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell*, 9, 435-43.

[54] Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM. (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A.*, 104, 15805-10.

[55] Chung HJ, Choi YE, Kim ES, Han YH, Park MJ, Bae IH. (2015) miR-29b attenuates tumorigenicity and stemness maintenance in human glioblastoma multiforme by directly targeting BCL2L2. *Oncotarget*, 6, 18429-44.

[56] Ng EK, Tsang WP, Ng SS, Jin HC, Yu J, Li JJ, Röcken C, Ebert MP, Kwok TT, Sung JJ. (2009) MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer. *Br J Cancer*, 101, 699-706.

[57] Braconi C, Huang N, Patel T. (2010) MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology*, 51, 881-90.

[58] Suzuki H, Maruyama R, Yamamoto E, Kai M. (2013) Epigenetic alteration and microRNA dysregulation in cancer. *Front Genet*., 4, 258.

[59] Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM. (2006) Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev*., 20, 2202-7.

[60] Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. (2004) The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev*., 18, 3016-27.

[61] Fukuda T, Yamagata K, Fujiyama S, Matsumoto T, Koshida I, Yoshimura K, Mihara M, Naitou M, Endoh H, Nakamura T, Akimoto C, Yamamoto Y, Katagiri T, Foulds C, Takezawa S, Kitagawa H, Takeyama K, O'Malley BW, Kato S. (2007) DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat Cell Biol*., 9, 604-11.

[62] Fuller-Pace FV, Ali S. (2008) The DEAD box RNA helicases p68 (Ddx5) and p72 (Ddx17): novel transcriptional co-regulators. *Biochem Soc Trans*., 36, 609-12.

[63] Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. (2007) Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet*., 39, 673-7.

[64] Musilova K, Mraz M. (2015) MicroRNAs in B-cell lymphomas: how a complex biology gets more complex. *Leukemia*, 29, 1004-17.

[65] Mori M, Triboulet R, Mohseni M, Schlegelmilch K, Shrestha K, Camargo FD, Gregory RI. (2014) Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. *Cell*, 156, 893-906.

[66] Kang W, Tong JH, Lung RW, Dong Y, Zhao J, Liang Q, Zhang L, Pan Y, Yang W, Pang JC, Cheng AS, Yu J, To KF (2015) Targeting of YAP1 by microRNA-15a and microRNA-16-1 exerts tumor suppressor function in gastric adenocarcinoma. *Mol Cancer*, 14, 52.

[67] Cochrane DR, Cittelly DM, Howe EN, Spoelstra NS, McKinsey EL, LaPara K, Elias A, Yee D, Richer JK. (2010) MicroRNAs link estrogen receptor alpha status and Dicer levels in breast cancer. *Horm Cancer*, 1, 306-19.

[68] Martello G, Rosato A, Ferrari F, Manfrin A, Cordenonsi M, Dupont S, Enzo E, Guzzardo V, Rondina M, Spruce T, Parenti AR, Daidone MG, Bicciato S, Piccolo S. (2010) A MicroRNA targeting dicer for metastasis control. *Cell*, 141, 1195-207.

[69] Inoue T, Iinuma H, Ogawa E, Inaba T, Fukushima R. (2012) Clinicopathological and prognostic significance of microRNA-107 and its relationship to DICER1 mRNA expression in gastric cancer. *Oncol Rep*., 27, 1759-64.

[70] Luo Z, Dai Y, Zhang L, Jiang C, Li Z, Yang J, McCarthy JB, She X, Zhang W, Ma J, Xiong W, Wu M, Lu J, Li X, Li X, Xiang J, Li G. (2013) miR-18a promotes malignant progression by impairing microRNA biogenesis in nasopharyngeal carcinoma. *Carcinogenesis*, 34, 415-25.

[71] Rubenstein JL, Shen A, Batchelor TT, Kadoch C, Treseler P, Shuman MA. (2009) Differential gene expression in central nervous system lymphoma. *Blood*, 113, 266-7.

[72] Montesinos-Rongen M, Brunn A, Bentink S, Basso K, Lim WK, Klapper W, Schaller C, Reifenberger G, Rubenstein J, Wiestler OD, Spang R, Dalla-Favera R, Siebert R, Deckert M. (2008) Gene expression profiling suggests primary central nervous system lymphomas to be derived from a late germinal center B cell. *Leukemia*, 22, 400-5.

[73] Rubenstein JL, Fridlyand J, Shen A, Aldape K, Ginzinger D, Batchelor T, Treseler P, Berger M, McDermott M, Prados M, Karch J, Okada C, Hyun W, Parikh S, Haqq C, Shuman M. (2006) Gene expression and angiotropism in primary CNS lymphoma. *Blood*, 107, 3716-23.

[74] Tun HW, Personett D, Baskerville KA, Menke DM, Jaeckle KA, Kreinest P, Edenfield B, Zubair AC, O'Neill BP, Lai WR, Park PJ, McKinney M. (2008) Pathway analysis of primary central nervous system lymphoma. *Blood*, 111, 3200-10.

[75] Camilleri-Broët S, Crinière E, Broët P, Delwail V, Mokhtari K, Moreau A, Kujas M, Raphaël M, Iraqi W, Sautès-Fridman C, Colombat P, Hoang-Xuan K, Martin A. (2006) A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. *Blood*, 107, 190-6.

[76] Braaten KM, Betensky RA, de Leval L, Okada Y, Hochberg FH, Louis DN, Harris NL, Batchelor TT. (2003) BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. *Clin Cancer Res*., 9, 1063-9.

[77] Jiang L, Marlow LA, Cooper SJ, Roemeling CV, Menke DM, Copland JA, Tun HW. (2010) Selective central nervous system tropism of primary central nervous system lymphoma. *Int J Clin Exp Pathol.* 3, 763-7.

[78] Visani M, Acquaviva G, Marucci G, Ragazzi M, Fraceschi E, Brandes A, Tallini G, Pession A, Biase D. (2015) MicroRNA in Brain Neoplasia: A Review. *Int J Brain Disord Treat.,* 1:002.

[79] Mazan-Mamczarz K, Gartenhaus RB. (2013) Role of microRNA deregulation in the pathogenesis of diffuse large B-cell lymphoma (DLBCL). *Leuk Res.,* 37, 1420-8.

[80] Zheng J, Xu J, Ma S, Sun X, Geng M, Wang L. (2013) Clinicopathological study of gene rearrangement and microRNA expression of primary central nervous system diffuse large B-cell lymphomas. *Int J Clin Exp Pathol*., 6, 2048-55.

[81] Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE. (2005) Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc Natl Acad Sci U S A.*, 102, 3627-32.

[82] Roehle A, Hoefig KP, Repsilber D, Thorns C, Ziepert M, Wesche KO, Thiere M, Loeffler M, Klapper W, Pfreundschuh M, Matolcsy A, Bernd HW, Reiniger L, Merz H, Feller AC. (2008) MicroRNA signatures characterize diffuse large B-cell lymphomas and follicular lymphomas. *Br J Haematol*., 142, 732-44.

[83] Malumbres R, Sarosiek KA, Cubedo E, Ruiz JW, Jiang X, Gascoyne RD, Tibshirani R, Lossos IS. (2009) Differentiation stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas. *Blood,* 113, 3754-64.

[84] Iqbal J, Shen Y, Huang X, Liu Y, Wake L, Liu C, Deffenbacher K, Lachel CM, Wang C, Rohr J, Guo S, Smith LM, Wright G, Bhagavathi S, Dybkaer K, Fu K, Greiner TC, Vose JM, Jaffe E, Rimsza L, Rosenwald A, Ott G, Delabie J, Campo E, Braziel RM, Cook JR, Tubbs RR, Armitage JO, Weisenburger DD, Staudt LM, Gascoyne RD, McKeithan TW, Chan WC. (2015) Global microRNA expression profiling uncovers molecular markers for classification and prognosis in aggressive B-cell lymphoma. *Blood*, 125, 1137-45.

[85] Fischer L, Hummel M, Korfel A, Lenze D, Joehrens K, Thiel E. (2011) Differential micro-RNA expression in primary CNS and nodal diffuse large B-cell lymphomas. *Neuro Oncol*., 13, 1090-8.

[86] Zhang J, Jima DD, Jacobs C, Fischer R, Gottwein E, Huang G, Lugar PL, Lagoo AS, Rizzieri DA, Friedman DR, Weinberg JB, Lipsky PE, Dave SS. (2009) Patterns of microRNA expression characterize stages of human B-cell differentiation. *Blood,* 113, 4586-94.

[87] Lawrie CH, Soneji S, Marafioti T, Cooper CD, Palazzo S, Paterson JC, Cattan H, Enver T, Mager R, Boultwood J, Wainscoat JS, Hatton CS. (2007) MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. *Int J Cancer*, 121, 1156-61.

[88] Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res*., 65, 9628-32.

[89] Rinaldi A, Poretti G, Kwee I, Zucca E, Catapano CV, Tibiletti MG, Bertoni F. (2007) Concomitant MYC and microRNA cluster miR-17-92 (C13orf25) amplification in human mantle cell lymphoma. *Leuk Lymphoma,* 48, 410-2.

[90] Mashima R. (2015) Physiological roles of miR-155. *Immunology,* 145, 323-33.

[91] Faraoni I, Antonetti FR, Cardone J, Bonmassar E. (2009) miR-155 gene: a typical multifunctional microRNA. *Biochim Biophys Acta*, 1792, 497-505.

[92] Jiang D, Aguiar RC. (2014) MicroRNA-155 controls RB phosphorylation in normal and malignant B lymphocytes via the noncanonical TGF-β1/SMAD5 signaling module. *Blood,* 123, 86-93.

[93] Robertus JL, Harms G, Blokzijl T, Booman M, de Jong D, van Imhoff G, Rosati S, Schuuring E, Kluin P, van den Berg A. (2009) Specific expression of miR-17-5p and miR-127 in testicular and central nervous system diffuse large B-cell lymphoma. *Mod Pathol*., 22, 547-55.

[94] Thapa DR, Bhatia K, Bream JH, D'Souza G, Rinaldo CR, Wolinsky S, Detels R, Martínez-Maza O. (2012) B-cell activation induced microRNA-21 is elevated in circulating B cells preceding the diagnosis of AIDS-related non-Hodgkin lymphomas., *AIDS*, 26, 1177-80.

[95] Jørgensen LK, Poulsen MØ, Laursen MB, Marques SC, Johnsen HE, Bøgsted M, Dybkær K. (2015) MicroRNAs as novel biomarkers in diffuse large B-cell lymphoma--a systematic review. *Dan Med J.*, 62, A5048.

[96] Shalaby T, Grotzer MA. (2015) Tumor-Associated CSF MicroRNAs for the Prediction and Evaluation of CNS Malignancies. *Int J Mol Sci*., 16, 29103-19.

[97] Wei D, Wan Q, Li L, Jin H, Liu Y, Wang Y, Zhang G. (2015) MicroRNAs as Potential Biomarkers for Diagnosing Cancers of Central Nervous System: a Meta-analysis. *Mol Neurobiol*., 51, 1452-61.

[98] Rao P, Benito E, Fischer A. (2013) MicroRNAs as biomarkers for CNS disease. *Front Mol Neurosci*., 6, 39.

[99] Jones K, Nourse JP, Keane C, Bhatnagar A, Gandhi MK. (2014) Plasma microRNA are disease response biomarkers in classical Hodgkin lymphoma. *Clin Cancer Res.,* 20, 253-64.

[100] Drusco A, Bottoni A, Laganà A, Acunzo M, Fassan M, Cascione L, Antenucci A, Kumchala P, Vicentini C, Gardiman MP, Alder H, Carosi MA, Ammirati M, Gherardi S, Luscrì M, Carapella C, Zanesi N, Croce CM. (2015) A differentially expressed set of microRNAs in cerebro-spinal fluid (CSF) can diagnose CNS malignancies. *Oncotarget,* 6, 20829-39.

[101] Akers JC, Ramakrishnan V, Kim R, Phillips S, Kaimal V, Mao Y, Hua W, Yang I, Fu CC, Nolan J, Nakano I, Yang Y, Beaulieu M, Carter BS, Chen CC. (2015) miRNA contents of cerebrospinal fluid extracellular vesicles in glioblastoma patients. *J Neurooncol*., 123, 205-16.

[102] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. (2008) Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A.,* 105, 10513-8.

[103] Shi R, Wang PY, Li XY, Chen JX, Li Y, Zhang XZ, Zhang CG, Jiang T, Li WB, Ding W, Cheng SJ. (2015) Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients. *Oncotarget,* 6, 26971-81.

[104] Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS, Harris AL. (2008) Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol.*, 141, 672-5.

[105] Fang C, Zhu DX, Dong HJ, Zhou ZJ, Wang YH, Liu L, Fan L, Miao KR, Liu P, Xu W, Li JY. (2012) Serum microRNAs are promising novel biomarkers for diffuse large B cell lymphoma. *Ann Hematol.*, 91, 553-9.

[106] Baraniskin A, Kuhnhenn J, Schlegel U, Chan A, Deckert M, Gold R, Maghnouj A, Zöllner H, Reinacher-Schick A, Schmiegel W, Hahn SA, Schroers R. (2011) Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. *Blood*, 117, 3140-6.

[107] Baraniskin A, Kuhnhenn J, Schlegel U, Schmiegel W, Hahn S, Schroers R. (2012) MicroRNAs in cerebrospinal fluid as biomarker for disease course monitoring in primary central nervous system lymphoma. *J Neurooncol.*, 109, 239-44.

[108] Baraniskin A, Kuhnhenn J, Schlegel U, Maghnouj A, Zöllner H, Schmiegel W, Hahn S, Schroers R. (2012) Identification of microRNAs in the cerebrospinal fluid as biomarker for the diagnosis of glioma. *Neuro Oncol.*, 14, 29-33.

[109] Teplyuk NM, Mollenhauer B, Gabriely G, Giese A, Kim E, Smolsky M, Kim RY, Saria MG, Pastorino S, Kesari S, Krichevsky AM. (2012) MicroRNAs in cerebrospinal fluid identify glioblastoma and metastatic brain cancers and reflect disease activity. *Neuro Oncol.*, 14, 689-700.

[110] Mao X, Sun Y, Tang J. (2014) Serum miR-21 is a diagnostic and prognostic marker of primary central nervous system lymphoma. *Neurol Sci.*, 35, 233-8.

[111] Roth P, Keller A, Hoheisel JD, Codo P, Bauer AS, Backes C, Leidinger P, Meese E, Thiel E, Korfel A, Weller M. (2015) Differentially regulated miRNAs as prognostic biomarkers in the blood of primary CNS lymphoma patients. *Eur J Cancer*, 51, 382-90.

[112] Bak M, Silahtaroglu A, Møller M, Christensen M, Rath MF, Skryabin B, Tommerup N, Kauppinen S. (2008) MicroRNA expression in the adult mouse central nervous system. *RNA*, 14, 432-44.