microRNA expression in non-Hodgkin's lymphomas

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SUMMARY

microRNAs are a class of short, single-stranded, noncoding RNA molecules that negatively regulate the expression target mRNA at posttranslational level. microRNAs as key regulatory molecules play important biological function and might act as tumor suppressor oncogenes in cancer and lymphomas. microRNAs cause the expression of important cancer related genes and might prove useful in the diagnostics, prognosis, and treatment of some lymphomas This review focuses on the role of microRNAs in normal lymphocyte differentiation and in development of non-Hodgkin's lymphomas.

Key words: *MicroRNAs; Lymphoma; Lymphoma, Non-Hodgkin; Non MeSH Non-coding RNA; B-Lymphocytes; T-Lymphocytes; Cell Differentiation*

Arch Oncol 2008;16(3-4):59-68.

UDC: 616-006.44:376.385.4
DOI: 10.2298/AOO0804059B

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Abbreviations:
miR - microRNA; Ago - Argonaute; DGCR8 - Di George Syndrome Critical Region gene, Exp-5 - Exportin-5; RISC - miRNAs induced Silencing Complex, 3'-UTR - 3'-Untranslation Region, LNA - Locked Nucleic Acid, CLL - Chronic Lymphocytic Leukemia; DLBCL - Diffuse Large B-Cell Lymphoma; GCB – Germinal center B Cell; ABC – Activated B Cell; GC - germinal center; TU – Transcripion Units; FL - Follicular Lymphoma; MCL - Mantle Cell Lymphoma; MCL-BV – Mantle Cell Lymphoma – Blastoid Variant; BL - Bürkitt Lymphoma; SMZL – Spleen Marginal Zone Lymphoma; HPV - Human Papilloma virus; EBV - Epstein-Barr virus; CMV - Cytomegalo virus; MLV - Murina Leukemia Virus; MD - Marek's Disease Virus; ALV – Avian Leukosis Virus; PEL - Primary Effusion Lymphoma; TGF-β - Transforming Growth Factor β; ERK - Extracellulare Signaling Regulation Kinase; JNK, -c-JUN-N terminal kinase; AID - Activation Induced Cytidine Deaminase; TCR - T-cell receptor; Th - T-helper; ds RNA - double strand RNA; RAKE - Array-based - Klenov enzyme assay; DP - Double positive; CLP - common lymphocytic precursors

Received: 10.12.2008
Provisionally accepted: 14.12.2008
Accepted: 17.12.2008

INTRODUCTION

microRNAs (miRNAs) are recently discovered new regulatory class of short noncoding single-stranded RNAs of approximately 18 – 24 nucleotides (nt), they regulate post-transcriptional gene expression by imperfect base paring with complementary sequences location mainly, but not exclusively, in the 3' untranslated regions (UTRs) of target messenger RNAs (mRNAs) (1). microRNAs represent one of the major regulatory family of genes in eukaryotic cells that inhibit the expression of mRNAs by translation repression or/and destabilizing target transcript (2). microRNA alterations are involved in the initiation and progression of human cancer. The microRNA genes can function as oncogenes (miRNA-17-92, miRNA 155/BIC) or tumor suppressor genes (miR-15a/mir-16, and miR-143/miR-145). Frank Slack called miRNAs, which play role in causing cancer, "oncogenic microRNAs" or "oncomirs" and miR17-92 he named oncomir-1. The combination of genetic or epigenetic events could contribute to the down-regulation or overexpression of miRNAs (3). In the last three years, significant deregulation of miRNA expression in many human cancer and lymphomas was demonstrated. Translocation breakpoints, the inactivation of tumor suppressor genes, and abnormalities in the apoptotic pathways are associated with many types of lymphomas (4). Calin A et al., 2004 suggested that one or more tumor suppressing genes are involved in pathogenesis of B cell lymphomas. The application of microarray mir technology led to the discovery of miRNA genes expression signature for the major groups of non-Hodgkin's lymphoma. microRNA expression profiles could correlate with particular tumor phenotypes and can be used as a very accurate diagnostic tool for classification and prognosis of cancer or lymphoma (5). The expression profiles of microRNAs in CLL may be useful as prognostic signature or may predict outcome of disease on therapy (6). Nowadays, several research groups explored the potential of microRNA-based cancer therapy with antagomir that inhibits specific microRNAs (6,7).

The molecular classification of non-Hodgkin's lymphoma using miRNA microarray profiling is area of intense research. In this review, we summarize the recent advances on miRNA genes: their identification, biogenesis, function, and expression profiling in non-Hodgkin's lymphoma.

A BRIEF HISTORY OF microRNA DISCOVERY

The history of the miRNAs discovery is an interesting story. Alexander Rich (1961) gave a prophetic suggestion: "… a complementary second strand of RNA may be a part of the control apparatus for on or off the synthesis of the proteins. One might imagine that one RNA strand is continually used in protein synthesis, while the other strand is used to control the rate at which the gene strand works" (8). However, the story of microRNAs discovery begin with genetic studies at cell lineages and in C. elegans. Sydney Brevaer from Cambridge, middle 1970, discovered first heterochronous genes Lin-4 mutant in C. elegans. Later, Howard Robert Horvitz identified another mutant Lin-14 gene. The Ambros Victor group from MIT, 1993, identified the first known microRNA, the Lin-4, or "small temporal RNA", which regulates temporal control of post embryonic development changes in C. elegans, but the structure and limits of this gene were unknown at that time (9). At the same time, Gary Ruwkin discovered Lin-14 as first microRNA target gene (10). These two discoveries made by Ambros and Ruwkin groups (June 1992), showed a novel posttranscriptional mechanism in which Lin-4 negatively regulates Lin-14 translation by binding directly to its 3' UTR (11). After 7 years from discovery of Lin-4, Ruwkin group identified and cloned second microRNA, Let-7, that encodes a small RNA, which negatively regulates gene Lin-41. The Let-7 and Lin-41 and hbl-1 are evolutionary conserved in various organisms from protozoa to humans (12). Later, in "epistasis" study, Ambros identified that Lin-4 negatively regulates Lin-14 and Lin-28, and Lin-14 negatively regulates Lin-28, Lin-29. Lin-29 acivates larval-to-adult (L A) switch (13). In Ambros laboratory, Eric Moss (1997) showed that Lin-4 regulates Lin-28 by a 3' UTR element and that Lin-4 regulates more than one mRNA (14). In that way, central genetic dogma (Figure 1) was completed by discovering of posttranscriptional activity of microRNAs on mRNAs.

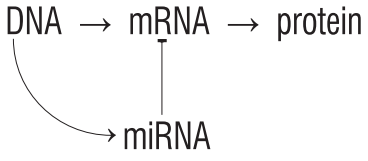

Figure 1. miR function in the negatively posttranscriptional regulation of gene expression

The rapid progression of research in microRNA area discovered more than 5000 of microRNAs in different prokaryotic and eukaryotic organisms. The microRNAs are discovered in Epstein-Barr virus, cytomegalo virus, Kaposi sarcoma herpes virus, SV-40, and HIV-1 virus.

In molecular researching of microRNA (from 1999 to 2001), exogenous dsRNA is identified by Dicer enzymes and conversion in mature miRNA molecules, and demonstrated miRNP complex by Nothern blot analysis (15).

In 2006, Nobel Prize for physiology and medicine was awarded to Andrew Z Fire and Crony C Mello for discovery of RNA interference gene silencing by double–strand RNA (dsRNA) (16).

In diverse organisms, microRNAs play roles in many processes from cell proliferation, apoptosis to hematopoiesis and cancer. The microRNA can function as a novel biomarker for disease diagnosis and is a new strategy for antagomir therapy (15,17).

BIOGENESIS AND FUNCTION OF microRNAS

Human miRNA genes are located in all chromosomes except Y chromosome and they are nonrandomly distributed in the genome. About 50% miRNAs are found in clusters and they are transcribed as polycistronic primary transcripts. Cluster mostly contains two or three genes. The largest cluster is composed of seven genes (18). Approximately 70% of mammalian miRNA genes are located in introns or exons. The 30% of miRNA gene are located in intergenic region and form independent transcription unit (TU) (19). For some human genes, more than one of microRNAs may be involved in their regulation. More than 80% of conserved miRNAs are tissue specific. Overall 50% of miRNA genes are in cancer-associated genomic regions or in fragile sites. Fragile sites are preferential sites of translocation, deletion, amplification, sister chromatid exchange (SCE), and tumor associated viruses (e.g. HPV, EBV, CMV).

Current estimates are that microRNAs genes represent about 1-5% of the predicted genes in humans and they control expression about 20-30% of human protein coding genes. They are involved in crucial biological processes, including the regulation of gene expression, cell proliferation and apoptosis, development, stress resistance, oncogenesis, renewal stem cells, fat metabolism and progression of many diseases (20). Today, in human genome more than 500 miRNAs have discovered (The miRBase server version 11 contiions 678 humna pre-miR and 847 mature miRNAs) (miRBase: http://www.sanger.ac.uk) but the estimated number of miRNAs genes is as high as 1000 (21).

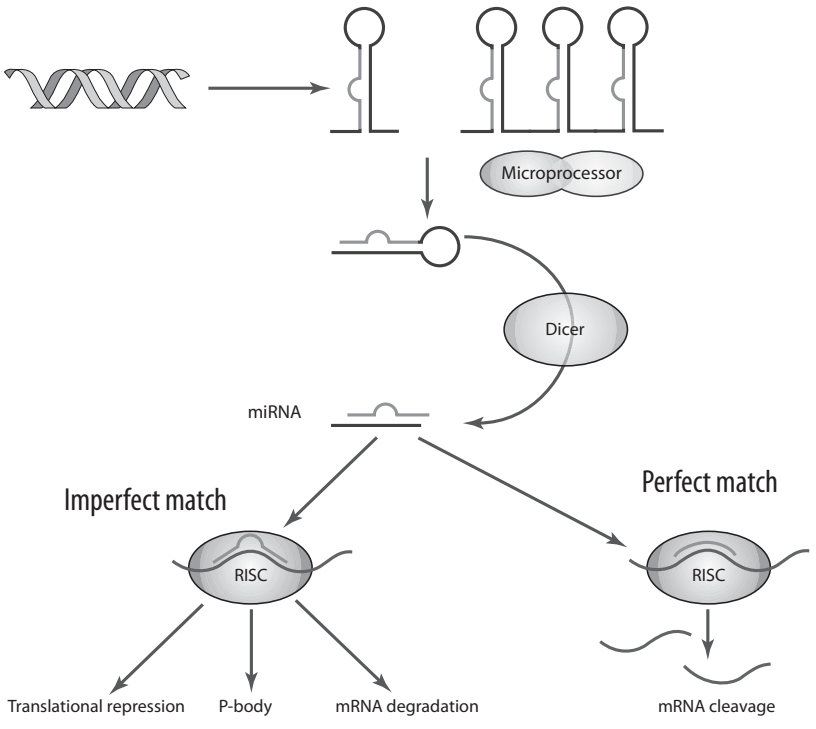

Figure 2. The biogenesis of microRNAs

The biogenesis of miRNAs begins with the transcription of miRNA genes by RNA polymerase II (Pol II) in the nucleus to generate large primary pri-miRNA transcripts, which are capped (MGpppG) and polyadenylated (AAAAA) (Figure 2). Regions of pri-miRNAs have short, imperfectly base-paired stem-loop structures containing sequences that will become the miRNA. These pri-miRNA transcripts processing begins with nuclear cleavage by the microprocessor complex, in which RNase III enzyme Drosha and its co-factor, Pasha or DGCR8 (Di George Syndrome Critical Region Gene 8), release the-70-nucleotide pre-miRNA precursor product with an imperfect RNA duplex structure (22). The pre-miRNAs are transported from nucleus to cytoplasm by Exportin-5 in a Ran-GTP manner and they form a nuclear heterotrimer. In addition, Exp-5 is important for stabilizing pre-microRNAs in the nucleus. However, in conversion pri-miRNA into pre-miRNA act RNA helicases hnRNPA1, p68, and p72 (23). In the cytoplasm, another RNase III enzyme, Dicer, generating a transient 22 nucleotide miRNA:miRNA*duplex or hybrids. This duplex is loaded into the miRNA-associated multiprotein RNA-induced silencing complex (miRISC), which includes the Argonaute (Ago) proteins, VIG, Fragile X-related protein, nuclease tudor SN, and various helicases (gemin 3 and gemin 4), and the mature single-stranded miRNA (24). Ago-2 protein is specifically responsible for RISC cleavage activity. Human Ago-2 protein possesses three residues (D597, D669 and H807), which have the catalytic function (25). The Ago-2 protein participates in the production of a new class small RNAs, Piwi-interacting RNAs. The Ago-2 binds ssRNA via their PAZ domains, which may have implications for prediction of miRNA targets. Argonaut 2 releases miRNA* and the remaining complex contains the mature miRNA. The mature miRNA then binds to complementary sites in the 3'-untraslated region of a targeted mRNA and negatively regulates gene expression. In case of base pairing between the miRNA and its target, Argonaut 2 cleaves the mRNA, leading to its destruction. The miRNAs that bind to mRNA target with imperfect complementarity block target gene expression at the level of protein translation or cause instability of the mRNA. A reduction or elimination of mature miRNA that functions as a tumor suppressor leads to tumor formation. The amplification or overexpression of miRNA that function as an oncogne also results in tumor formation (15,18-21,26).

The miRNAs act as gene regulators, which mediate through translation repression or mRNA degradation via binding of miRNAsd to sites in the 3'-UTR of protein-coding transcripts. Translation repression interaction depends on perfect base pairing between the seed region end of the miRNA and target site. Six nucleotide sequences of 2-8 positions at the 5' region, known as "seed matching" region are crucial in binding to the target mRNAs and can be predicted by searching for conserved matches to the seed region. microRNAs can inhibit transition initiation, specifically the function of the cap binding initiation factor eIF4E (27,28). The function of human miRNAs is largely unknown. However, many studies demonstrated important roles for miRNAs in animal development. miRNA target predictions suggest important roles for miRNAs in humans. In humans, at least 10% of the protein-coding mRNA might be conserved targets of miRNAs. Many miRNAs regulate others human genes that are involved in transcriptional regulation crucial for cellular functions, such as proliferation, apoptosis, or tumorgenesis. The genomic amplification or deletion is a major mechanism of activation of the oncogenic function of miRNA or inactivation of this tumor suppressor function (28,29).

METHODS FOR DETECTION AND ANALYSIS microRNAs

Nowadays, for profiling expression of miRNAs and their quantification to apply in practice several methodologies which include: Northern blot, Dot blot, cloning, RNase protection assay, real-time RT-PCR, oligonucleotide microarrays, LNA modified oligonucleotide probes, in situ detection, single molecule detection, array-based Klenow enzyme assay, and based-based hybridization by flow cytometric assay (29,30). Northern blot method is used in the quantification of miRNAs, but is relatively insensitive in measuring miRNA. The real-time RT-PCR method use a stem-loop reverse transcription primer to generate cDNA templates, which are then amplified using specific primers and detected with specific probes, similar to the standard RT-PCR. The most widely used method is based on microarrays. microRNA arrays are now applied to explore the biogenesis of miRNAs, molecular disease characterization, mechanisms of action miRNA and cancerogenesis, in evaluation of drug efficacy and toxicity, and stem cell development, etc. (31,32). Recently developed microarrays detect mature miRNA by employing antisense oligonucleotides that specifically bind to the mature miRNA sequences. The microarrays assays are well suited for large scale screening and are very sensitive. However, the use of locked nucleic acid (LNA) modified capture probes (miChip) has improved the ability of miRNA microarrays to measure more accurately levels of highly homologous miRNAs and dramatically reduces the time necessary for carrying out the protocol. The array-based Klenow enzyme assay (RAKE) used to profile miRNA expression from formalin-fixed tissues samples for advancing miRNA research. The most recent innovation in miRNA detection surrounds the based-based flow cytometric method (30,32-35).

microRNAs IN LYMPHOCYTIC DEVELOPMENT

The lymphocytic system offers an ideal model for studying of miRNA in lymphocytic maturation. miRNAs control differentiation of regulatory T-helper cells and the germinal center reaction in T cells dependent antibody response (36). microRNAs have important role in regulation of normal lymphocyte and lymphoma development. In complex network regulation, function of microRNAs is largely unknown, but its function through transcription factors has been understood to a certain degree (37). The activity of several microRNAs has been described in lymphopoiesis so far, for example micro-181a -155, 150 (Figure 3).

microRNA-181a is highly expressed in thymus and other hematopoietic tissue, and in brain and lung. miR-181a is downregulated in CD4+ lymphocytes and other tissues and inhibits production of CD8+ lymphocytes. miR142s inhibits production of CD4+ and CD8+ T lymphocytes and does not affect B lymphocytes production (38). In this differentiation, miR-181a acts to target Bcl-2, CD69, and TCRα. In addition, Mir-181a in Dicer deficient mice causes downregulation of BCl-2 expression (39). Ablation of Dicer and loss of mature miRNAs in embryonic fibroblast upregulate p19 ARF and p53 levels and cause inhibition cell proliferation. When miR-181a is ectopically expressed in hematopoietic progenitor cells, it regulates B and T lymphocytes development (40). miR-181a is promoter or positive regulator of B lymphocyte differentiation through the repression of Notch signal pathway, but it can also modulate TCR sensitivity and signaling strength (41). Mir-181a causes high expression of CD4+CD8+ in double positive (DP) stage of T lymphocyte development, and lower expression of CD4+CD8+ in the mature Th1 and Th2 cells that are only reactive to relatively high affinity for foreign antigens. In T cells differentiation, microRNA-181a increases sensitivity of mature T lymphocytes to antigens by modulation of expression of costimulating molecules CD28 and CTLA-4 (41,42). Downregulation of miR-181a in immature T lymphocytes reduces their sensitivity and impaired T cell selection, through downregulation of phosphatases genes (SHP2, PTNP22, DUSP5, DUSP6) (40,42,43). The antagomir may, by inhibition of miR-181a in double positive T lymphocytes, to impair sensitivity to antigens and to inhibit positive and negative selection in the stages T lymphocyte development (42-44).

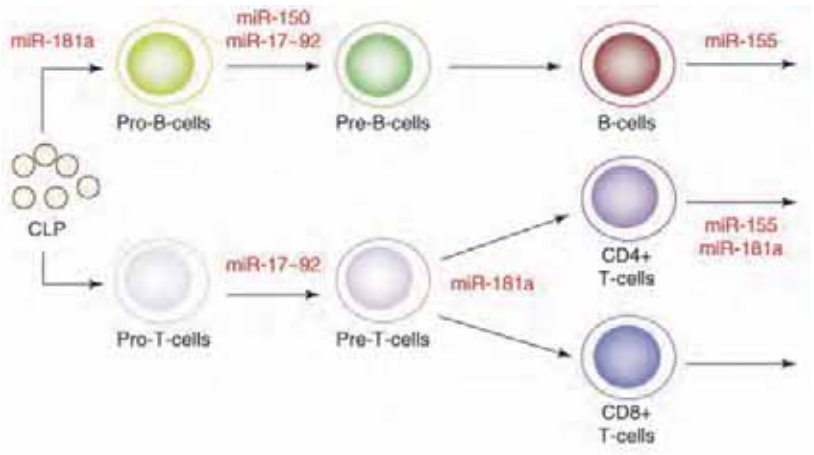

Figure 3. microRNAs and its role in lymphocytic development

The BIC/miR-155 plays a key role in regulation of cytokine, chemokines, and transcription factor genes, which control effector function of the immune system and hemostasis function (45). The BIC/miR-155 expression is increased in activated B and T lymphocytes, macrophages, and dendritic cells (43,46,47). The miR-155 is critical for B cells maturation and immunoglobulin production in response to antigen. The miR-155 upregulates BCR and TCR and plays role in germinal center formation. The BCR activation results in the induction of c-fos, FOS-B, and JUN-B through ERK (extracellular signaling regulated kinase), and JNK(c-JUN N terminal kinase) pathways. In the miR-155 deficient mice, immunization with antigens causes downregulation of IgG1 response; production of IL-2 and INFgama is reduced and dendritic cells are unable to stimulate T-cell proliferation. The antigens activate follicular B cells and reduce extrafollicular and germinal center responses. About 60 genes participating in B lymphocyte differentiation has been identified so far. However, PU.1 is a direct target gene for miR-155. miR-155 is responsible for normal production of high affinity antibodies and memory response and for activation of cytidine deaminase (AID) gene (48). In the BIC-miR-155 CD4+ deficient cells, a wide spectrum of miR-155 regulated genes is identified including genes for cytokine, chemokine, and transcription factors (47,48). In maintaining the mature normal B cell phenotype and malignant B cells important role is played by miR-223 and its transcription factors LOM2, Mybl, and by miR-29, -30 and its transcription factor Blimp-1 (49). The Blimp-1 is a regulator of terminal differentiation of B cells in antibody secretory plasma cells, and regulator of T cells differentiation (50,51).

In the mature T cells there is global downregulation of miRNAs (miR-16, 21, -142-3p, 142.5p, 150, -156, and let -7f) (37,52).

In the Dicer deficient T cells, a block in development of CD8+ peripheral lymphocytes and reduced number of CD4+ cells was identified (52,53).

microRNA-150 plays role in differentiation of B lymphocytes that are in transition stage from pro-B cells into pre-B cells. In experiments on mice, it was identified that the ectopic expression of miR-150 in hematopoietic stem cells causes reduced differentiation of mature B cells (54). Exact mechanism of this B cell downregulation is inhibition of transition from pro-B to pre-B cells through the fine tuning of c-myb expression during B cells differentiation. In addition, miR-150 in response to T cells stimulation downregulates Th1 or Th2 lymphocyte subsets (42,52,54,55).

The miR-17-92 cluster and its three members: miR-17, miR-20b, and miR-106a, are critical to the normal development of lung cells and B cells. In these cells, miR-17-92 cluster suppresses gene that induces cell death (55). Karlov et al., and Ventura et al., discovered that deficiency of miR17-92 or Dicer molecules in pro-B cells stops differentiation to pre-B cell (55). The exact genes targeted by miR17-92 are not known, but suspect is PTEN and Bim gene, which promotes cell death (55,56). The miR142s inhibits production of both CD4+ and CD8+ lymphocytes (56) but does not affect B cells. The Aiolos is member of the Ikaros family of zinc finger proteins, which plays role in the control of mature B lymphocytes differentiation and proliferation (38,57,58).

microRNA IN LYMPHOMAGENESIS

The alterations in the expression of several microRNAs in cancer and lymphomas suggested potential roles of miRNA in lymphomagenesis. More than one-half of the microRNAs genes are located in cancer-associated genomic regions. The global decrease of miRNAs expression in tumor cells or overexpression might contribute to the generation and maintenance of tumor cells and cancer steam cells. miRNAs can control expression of target oncogenes and tumor suppressor genes (Table 1, Figure 4) (59).

Table 1. Most frequently deregulation miRNAs their functions and target genes in non-Hodgkin lymphoma

microRNA	Location / host gene cancer condition	Expression	Identified targets	Function OG or TSG	Reference
miR-15a/-16-1	13q14.3-D, D139272-D13925, deletion intron 4 of DLEU2	↓CLL	BCL-2	TSG	1, 22, 29, 33, 60, 61
miR-155	BIC-21q21	↑DLBCL(ABC), ↑CLL, L, ↑FL, ↑PMBL, ↑PTLD, ↑pediatric BL, ↓adult BL	ATR1, FADD, IKKε, Ripk, TP53, MMP-3, BACH1, PU.1	OG	22, 29, 56, 58, 64, 66, 77, 88, 90
miR-17-92 cluster (miR-17, -18, -19a, -20, -19b1, -93-1)	(C13 of f25) amplification (13q31-32, intron 3) 13q32-33-A, stSG15303-stSG31624	↑DLBCL, ↑BCL, ↑MCL ↑FL	PTEN, Bim E2F1→E2F3, cooperate with c-myc, E2F1 (miR17-5p, miR-20), TGFβ RII (miR-20)	OG/TSG	18, 22, 29, 33, 70, 73, 90,
miR-21	17q23.2, 3'-UTR	↑DLBCL(ABC), ↑CLL	PTEN, BCL-2, TPM1, Pdcd4	OG	29, 77
miR-221	VMP1	↑DLBCL(ABC), ↑FL	c-kit, p27kipP1	OG	89
miR-29	FRA7	↑CLL, ↓SMZL	Tcl1, DNMT3A, DNMT3B	OG/TSG	82
miR-143/-145	5q32-33, intergenic	↓DLBCL(ABC), ↓CLL, ↓BCL, ↓BL, ↓MALT	ERK-5	TSG	22, 83
miR-181a	NRGA1	↑CLL	Tcl1, HOX-A11, BCL-2, DUSP-6, SHP2, PTPN22	OG/TSG	44, 58, 77
miR-181a'		↓CLL[x]			[x]
LET 7a↓		↓CLL[*] ↓CLL[x]			[*], [x]
UC.73A(P)	BC017741	↓CLL		TSG	[x]
UC.135(E)	EVI1	↓CLL		TSG	[x]
UC.233(E)	centG3	↓CLL		TSG	[x]
UC.291	corf11	↓CLL		TSG	[x]
BART2 miR		↑EBV, ↑PELs			96
BART2-BHRF1	CXCL-11/TAC	↑DLBCL			96

Abbreviations: CLL – chronic lymphocyte leukemia, DLBCL – diffuse large B-cell lymphoma, BCL – B-cell lymphoma, FL – follicular lymphoma, PMBL – primary mediastinal B-cell lymphoma, PTLD – post-transplantation lymphoproliferative disorder, PEL – primary effussion lymphoma, BL – Bürkitt lymphoma, SMZL – splenic marginal zone lymphoma, MALT – mucosa-associated lymphoid tissue, OG – oncogene, TSG – tumor suppressor gene, DUSP – dual-specificirty protein phosphatase, FADD – Fas-associated death domain, IKKε – IkBkinase, PTEN – phosphatase and tensin homolog, PTPN22 – protein tyrosine phosphatase, nonreceptor-22, Ripk – receptor interacting protein kinase, SHP2, SH2 – domain-containing protein tyrosine phosphate, TP53 – tumor protein 53, Bim, BCL-2-like 11

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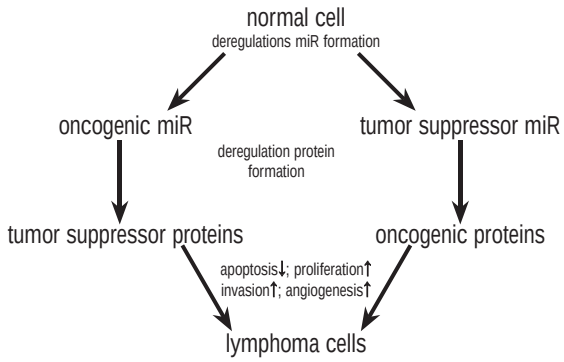

Figure 4. Function of microRNA in lymphomagenesis

Calin et al. (2000) were the first to identify miR-15a, and miR-16-1, which are downregulated by hemizygous or homozygous deletion at 13q14 chromosome in 68% of chronic lymphocytic leukemia (CLL). In one case of family history of CLL, a point mutation in precursor molecule for miR-16-1 was identified. Reduction of miR-15a and miR-16-1 levels was observed in about of 50% cases of homozygous deletion 13q14. The germ-line or somatic mutations might play role in cancer development (60). The miR-15a and miR-16-1 may function as tumor suppressor genes, which are located at 13q14 chromosome. These miRNAs negatively regulate the expression of the antiapoptotic Bcl-2 gene. Thus, downregulation of miR-15a and miR16-1 could result in increased Bcl-2 protein level and anti-apoptotic activity (61). The miR-15a and miR-16-1 are natural antisense Bcl-2 interactions and could be used for the therapy of some tumors (62).

The noncoding RNA gene BIC was originally identified as a proviral insertion site in avian leucosis virus (ALV) which induced B cell lymphomas in mouse *c-myc* model (63). The phyllogentically conserved region at the C-terminal exon in the BIC gene encoded a functional precursor of miR-155 (64). Costinean et al. (2006) generated transgenic mice that overexpressed *c-myc* carrying the miR-155 precursor sequence with B cell targeted expression. They identified that transgenic mice developed pre-B cell proliferation in spleen and bone marrow and eventually developed frank high grade B cell lymphoma after 6 months (65). BIC might function as a pri-microRNA; it is locked in the nucleus where it is processed into pre-microRNA. The nuclear localization of BIC transcripts is low in normal lymphoid tissue, but elevated in Hodgkin's lymphoma, primary mediastinal lymphomas, and DLBCL. Overexpression of miR-155 was detected in Burkitt lymphomas and post-transplantation lymphoproliferative disease when associated with EBV latency type III infection (66). In transgenic mice miR-155 may induce polyclonal B cell proliferation. Overexpression of miR-155 in mouse hematopoietic stem cells causes myeloproliferative disease. microRNA-155 BIC acts on the transcriptional factors PU.1 and C EBP β, which control B cell differentiation (67).

Another miRNA oncogene is miR-17-92 cluster or Oncomir-1 located at 13q32-33 in intron 3, which is amplified in human B cell lymphomas, lymphoma cell lines, lung cancer, and induced T lymphoma. (Figure 5) In experimental B-cell model, miR-17-92 cluster together with c-myc oncogene cooperates in induction of lymphoma progression and apoptosis inhibition (68). The miR-17-92 cluster induces overexpression of c-myc, and it is a target for *c-myc*. Transcriptional factor E2F1 is a target for miR-17-92 cluster. This feedback mechanism may help prevent accumulation of excessive amounts of E2F1. *c-myc* initiates apoptotic tumor-suppressive response, while E2F1 induces p21 protein resulting in S phase arrest of cell cycle (Figures 5,6).

The complex cyclin D1/miR-17/20 function as regulatory feedback loop. cyclin D1 induces miR-17-5p/miR-20a that limits the proliferative function cyclin D1. miR-17-92 regulates the CDKN1B; hasa-miR-17-5p regulates E2F1-3, NOOA3, and RBL-2; miR-17-5p and miR-20a regulate PACF, RUNX-1, and TGF-βR2. Many of these targets are cell cycle regulators, e.g. miR-18a regulates CTFG gene, and miR-19a acts on PTEN and THBS-1. miR-17-5p is efficient decouple negative regulator of MAPK signaling cascade (Figures 5,6).

The inhibition of miR-20a by antisense oligonucleotide causes increased apoptosis after therapy with doxorubicin. miR-17-92 cluster may promote neovascularization through downregulation of the trombospondin-1 and connective tissue growth factor (69-71). In the transgenic mice, the miR-17-92 cluster suppresses expression of the tumor suppressor PTEN gene and the pro-apoptotic Bim gene (Figures 4,5).

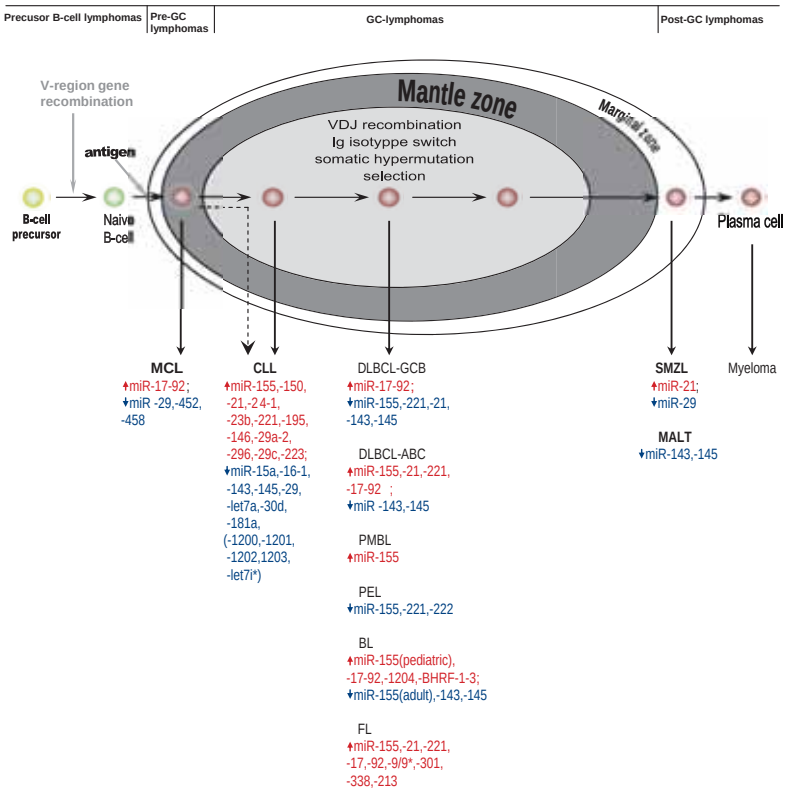

Figure 5. Aberrant expression of microRNAs in lymphomas (Abbreviations: see legend of Table 1)

ABERRANT EXPRESSION OF RNAs IN LYMPHOMAS

Chronic lymphocytic leukemia

miRNA expression genes correlate with various lymphomas. These genes function as both tumor suppressors and oncogenes. Chronic lyphomocytic leukemia (CLL) is biologically heterogeneous a B cell lymphomas with variable clinical course. In some patients, CLL is indolent disease and in others is more aggressive with poor prognostic outcome. CLL is characterized by accumulation of monoclonal CD5 B mature lymphocytes (74). In CLL, deletion of 13q14

is the most frequent genetic abnormality and very often the sole genetic alteration. Deletion of 13q14 is heterogeneous and composed of multiple subtypes, with deletion of RB or the miR-15a and miR-16-1 (Figure 5). Deletion type Ia is relatively uniform in length and extend breakpoints class to the miR-15a and miR-16-1 (75). Calin et al. demonstrated for the first time that deletions or downregulation of miR15a and miR16-1 gene are located at chromosome 13q14. Deletions of this locus occur in more than 65% of cases of chronic lymphocytic leukemia, as well as in 50% of mantle cell lymphoma, 16-40% of multiple myelomas, and 60% of prostate cancer (Table 1) (60,75). Calin et al. also determined the specific signature associated with expression of mutated IgV_H, a favorable prognostic marker in CLL. The miR186, miR-132, and miR-16-1 are positively associated with unmutated IgV_H, while miR-102, and miR-29-c are positively associated with mutated IgV_H. The miR-15a, and miR16-1 map within the intron of a non-protein-coding RNA gene of unknown function, which is called LEU2 (76-78). miR-16-1 regulates G0/G1 progression cell cycle. The expression signature composed of 13 miRNA genes could distinguish CLL cases with a poor prognosis and high ZAP-70/unmutated IgV_H phenotype from those CLL with favorable prognosis and low ZAP-70/mutated IgVH. The miRNA signature included high expression of miR-15a/miR-16-1, miR-24-1,/miR-23b, miR155, miR-221, miR-195, and miR-146 and low expression of miR-29a-2, miR-296, miR-29c, and miR-223 (Figure 5) (79). The miR-21 and miR-155 are dramatically overexpressed and miR-150 and miR-17-92 are significantly deregulated in patients with CLL (80). Cimmino and Calin et al. reported that miR15a and miR16-1 expression in CLL inversely correlated to expression of anti-apoptotic BCL2 gene and both miRNAs are negative regulators of BCL2 at a posttranscriptional level (81). The first 9 nucleotides from 5' of miR-15a and miR-16-1 are complementary to bases 3287 to 3279 on the 3'UTR of BCL2, which encodes a protein that can inhibit apoptosis. These miRNAs control the level of BCL2 in normal cells also but the high expression of the oncogenes lead to CLL (74,81). The latest report of Calin's group is about two CLL patients carried a germline C to T mutation of the miR-16-1 precursor, which results in decreased miR-16-1 expression levels. This mutation is associated with a family history of CLL (81). The aggressive form of CLL is characterized by low expression of miR-29 and miR-181b and high expression levels of Tcl-1 and MCL-1 genes (82). Also, a similar finding is identified in case of splenic marginal zone lymphomas. The expression of miR-143 and miR-145 decreased in CLL (83).

Diffuse large B cell lymphoma

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma. This lymphoma is heterogeneous genomic entity. By gene expression and immunochistochemical methods, three distinct subgroups of DLBCL were identified: GCB, ABC and PEL. The first subgroup showed as a germinal center B-cells (GCB) (CD10, BCL6, A-MYB, OGG1), This subgroup showed a frequent translocation t(14;18) involving the BCL2 gene. The second subgroup revealed activated B-cell like (ABC) with t(14;18) and Ig hypermutation. The third subgroup identified as primary mediastinal large B cell lymphoma is characterized by anatomic location, clinical presentation, histological and immunophenotypic features (CD30+, CD20+, CD79a+, Pax 5+, PU.1, OCT-2+). The GCB and ABC have different 5-years survival rates of 70-80% and 30-40%, respectively, when not treated with Rutiximab (84). Aberrations of microRNAs genes are widespread in primary of DLBCL and cell lines (85). About 95% of tumors and 100% cell lines have one alteration of miRNAs integrity. In 20% of DLBCL cases amplification of 36 microRNA genes and deletion in 10% miRNA genes was found (Figure 5) (86).

The miR-155 encoded by BIC nucleotides 241-262 was originally identified as a transcript derived from an integration site for the avian leucosis virus and found to be overexpressed in B cell lymphomas. Philogenetically conserved regions in BIC have the highest homology in 138 nucleotides section of the gene, which encoding the pri-miRNA for miR-155. Until now, 42 genes have been identified, which correlated inversely with miR-155 expression. Putative target genes for miR-155 are: MWAB, TIPI2DA, HNRPA2B1, PU.1, DCX, ENST00000296490, IDN3, OLFM3, BCROL-1, AP3D1, YPEL5, ENST 00000285951, ENST00000366942, DDIT4, MECP2, JARID2, TBR1, ZIC-3, ISCOL, GAB-2, ATP1A2, POLQ, BC029985 (87). The miR-155 acts as oncogne, but mechanism of action is unknown. BIC gene activation can accelerate the pathogenesis of lymphomas. The cells of DLBCL have 30–fold increase in copy numbers of miR-155 then normal circulating B cells (88). The BIC/miR-155 is positive in ~67% diffuse B cell lymphoma cases and primary mediastinal lymphoma. The miR-155 levels were elevated 12 to 60 fold relative to the control B cells in DLBCL. The miR-155 levels in ABC subgroup of DLBCL were two to three folds higher than in GCB-DLBCL subtype. The alterations in miR-155 expression are not absolutely specific for lymphomas (Figure 5). This deregulation is observed and in other solid tumors (29,88). Zhang B, Farwely MA, concluded, that miR expression signature which contain three miRNAs (miR-21, miR-155, and miR-221) was highly expressed in ABC subtype then in GCB-DLBCL type of DLBCL and may be used for distinguishing of these two types of DLBCL (Table 1). The expression of miR-21 was an independent prognostic factor in *de novo* DLBCL but no association between expression levels of miR-155 and miR-221 and DLBCL prognosis was found (89). The Smad proteins controlled the processing of miR from pri-miR-21 to pre-miR-21. The miR-221 is not a target for c-kit gene. The circulating levels of miR-155 were higher in serum patients with DLBCL compared to healthy control. The high expression of pre –miR-155/BIC RNA was detected in DLBCL, Burkitt's lymphoma, and other lymphomas (Table 1) (89,90).

Lam T et al, defined the signature with IL-6, IL-10, and STAT 3 to distinguish subset of ABC-DLBCL from other lymphoma subtypes (91).

Also, miR-17-92 cluster or homologous clusters miR-106a-92 and miR-106b-25 are overexpressed in DLBCL, lung cancer, and other solid tumors (92). Roehl et al., 2008, identified abnormal expression of miR-150, miR-17-5p, miR-145, and miR-328 (93). The miR-17-92 polycistron is involved in control of cell proliferation and angiogenesis (Table 1) (93,94).

Primary effusion lymphoma (PEL) is a rare DLBCL form, which is caused by Kaposi sarcoma herpes virus (KSHV) and in 80% of cases associated with EBV infection (Figure 5). KSHV encodes 12 microRNAs. The KSHV-miR-K12-11 is an ortolog has miR-155 and may contribute to the development of PEL's cell phenotype, which is a late stage of B cell differentiation (95). The EBV+ PEL cells and Burkitt's lymphoma cells (BLs) type I expressed only BART2 microRNA. Whereas, DLBCLs cell lines expressed BART2 microRNA and BHRF1-3 microRNAs. This expression inversely correlated with target CXCL-11 1TAC gene (96). In KSHV, infected lymphoma tumor specific downregulation of miR-155, miR-221, and miR-222 was identified (Table 1) (97).

Mantle cell lymphoma

Mantle cell lymphoma (MCL) represent 3-10% of all non-Hodgkin lymphomas, The MCL is considered a distinctive type of mature B cell lymphoma with aggressive clinical behavior. The MCL cells bear surface immunoglobulin and are often CD5+ cells. The genetic hallmark of MCL is a translocation t(11;14)(q23;q32), which results in ectopic up-regulation of cycline D1 in tumor cells. Translocation alters miR-16-1, and influences alteration in cycline D1 mRNA expression.

The CDN1A1/p21, Bcl-2, and myc are essential targets of miR-17-92 cluster in lymphomagenesis (Figure 6) (98).

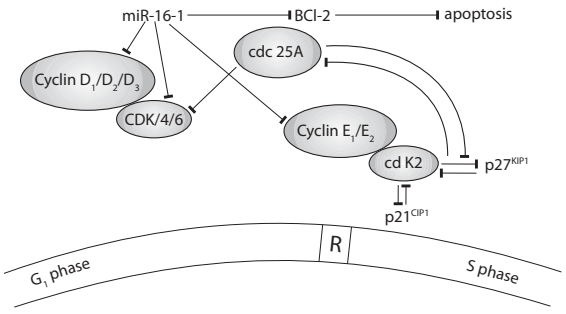

Figure 6. Function of miR-16-1 on cell cycle

The main morphological MCL subgroups are classical MCL and blastoid variant (BV), which constitute about 10% of MCL and is clinically and are more aggressive. In MCL-BV, there is increased number of numerical chromosomal alteration and more frequently losses of tp53 gene (99). By clustering analysis and principal component analysis of set of 15 miRNAs may differentiate conventional from blastoid form of MCL and progressive disease from non-progresive disese (Table 1) (98,99). Chen et al. identified a mutation in the 3'UTR of Syk at the docking site of 1 miR-452* and SNP in miR-458, which leads in downregulation of the Syk protein and progression of this disease (100). Downregulation of 18 microRNAs was identified in MCL. The expression of miR-29 families (miR-29a,b,c) inversely correlated with survival. Therefore, miR-29 is a new biomarker for MCL prognosis (Figure 5) (101).

Follicular lymphoma

Follicular lymphoma represents ~22% of all cases of non-Hodgkin's lymphomas and is typical indolent but incurable disease. Follicular lymphoma is B cell malignancy and has the tendency to transform into DLBCL, with translocation t(14;18)(q23;q21) in 90% of cases. This translocation leads to the overexpression of BCL2 gene. This transformation may be associated with gene rearrangement of *c-myc* gene, mutation in p53, mutation in BCL6, or inactivation of p15 or p16 (102). The t(14;18) translocation in 80-90% of FLs and consecutive overexpression of BCL-2 leads to an accumulation in GCB cells with prolonged life span. A later, during multistep process of lymphomagnesis additional genetic or epigenetic mutation lead to progress of FLs (103). Globally in low grade FLs significantly reduced expression of 38 microRNAs during transformation was detected. Reducted expression in miRNAs contributes the increase of expression oncogenes and transformation process of FLs in DLBCL (Table 1). In follicular lymphoma specific aberrant expression of MIRN9/9*, MIRN301, MIRN338, MIRN213 was identified. In addition, the miR-17-92 cluster is amplified in follicular lymphomas, Mantle cell lymphomas and primary cutaneous B cell lymphoma (Figure 5) (77,93,103). Specifical lymphoma form is spleen marginal zone lymphoma (SMZL). In this lymphoma miR-21 is overexpressed (Figure 5, Table 1) (104).

Burkitt's lymphoma

Burkitt's lymphoma (BL) is rare, highly aggressive B cell lymphoma that accounts for 30-50% of lymphomas in children but only 1-2% of lymphomas in adults. This lymphoma is characterized by high degree of proliferation of malignant cells and deregulation of the c-myc gene and their kappa and lamda chain Ig genes. The translocation of the myc and its consequente deregulation is a key oncogen event in the pathogenesis of BLs and in 5-10% of DLBCL. These both lymphomas originate from germinal center B or t(8;22) c-myc and PVT1 cells (105,106). In classic translocation t(8;14) and in variant translocation, t(2;8) or t(8;22), PVT1 gene product has-miR-1204 activates *c-myc* oncogene (107-109). Overexpression of RBL2 p130 is identified in BLs, which modulates the expression of target genes important for cell growth (CGRF1, RGS1, T1A1, and PCDHA2) (110). Xia et al. identified markedly elevated expression of miRNA BHRF1. In type III pediatric BL and in type I BL and primary effusion lymphomas high levels of BART2 miRNA are discovered. In all BL cases, BHRF1-3 miRNA expression inversely correlates with levels of a cellular target CXCL-11/1-TAC (96,111,112). The expression of BIC and miR-155 is not a common finding in BL (Table 1). However, EBV latency type III infection controls induction of BIC expression in EBV-positive BL cells lines and all posttransplantation lymphoproliferative disorders. In Ramos cell line, which originates from BL, a specific block in maturation of BIC to miR-155 was identified or processing of BIC *via* unknown mechanism was prevented. Partially reconstructive BIC activation and NF-kB activation cause expression of EBV-LMP1 that lead to immortalization of B lymphocytes (113-115). The expression levels of miR-143 and miR-145 were significantly reduced in the EBV transformed B cell lines, BL cell lines, CLL, and B cell lymphomas (Table 1, Figure 5). One target gene for miR-143 is ERK5 and for miR-145 are MAP3K3 and MAP4K4, and others. miR-143 and miR-145 may be useful as biomarkers of transformation normal cells to B neoplastic cells (83,116,117). In the clinical practice, the micro RNA expression profiles can be used to distinction between DLBCL and BLs (Figure 5).

T-cell lymphomas

The T cell lymphomas represent heterogeneous subgroups of non-Hodgkin's lymphomas. In these lymphomas role and function of microRNAs is at the beginning of research. In experimental model of T-cell lymphomas, caused by strain SL3-3 of murine leukemia virus (MLV), overexpression of the mmu-miR-1204 and miR-106a was identified (109,118). Also, Marek Disease Virus (MDV), which causes avian T cell lymphoma, play important role through expression of microRNA (118). In a peripheral T-cell lymphoma that is consistent with a type II latency EBV infection specific expression of miR-143 and miR-145 was identified (Table 1) (119).

CONCLUSION AND PERSPECTIVES

Discovered 16 years ago in C. elegans, many miRNAs are involved in a wide variety of physiological processes including embryogenesis, differentiation, proliferation, apoptosis, organogenesis, growth control, metabolism, carcinogenesis, etc. microRNAs are small single strand RNA molecules of 18 to 25 nucleotides in length that regulate the expression of other protein-coding genes

through an intracellular gene silencing mechanism, namely RNA interference. miRNAs are specific and important components differentiation of lymphocytes. The research of miRNAs alterations and cancer and lymphomas may lead to establishment of novel methods for precisely subclassifying some lymphomas based on miRNAs profiles. The combining miRomics, genomics, and proteomics might help delineating spectrum of targets that are regulated by miRNAs. Also, miRNAs may be used as a specific molecular marker for the stratification of prognostic subgroups of lymphomas. microRNAs are relatively resistant to RNase degradation and because of that useful for early detection, diagnosis and follow-up using biological fluids of lymphoma patients.

In future, it is necessary to continue work on determination of microRNA in stem cell maintenance, embryonic development, tissue differentiation, adult physiology, disease pathology, cancer research, and viral infection.

microRNAs are small molecules, may modulate multiple oncogenic pathways in cell, and may serve as potential effective targets for antitumor therapy. Nowadays, attention has been focused on study of antisense oligonucleotide to inhibit mRNA function.

Acknowledgments

I thank to Gordana Bogdanović for suggestions and additions in final version of the manuscript, and Stanka Matejašev, Tibor Lakatos and Sabine Terzić for technical assistance in manuscript realisation.

Presented at 12th Studenica meeting "Advances in Clinical Oncology", Studenica Monastery, Serbia, June 5-7, 2008.

Conflict of interest

We declare no conflicts of interest.

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