Karlova univerzita v Praze, Přírodovědecká fakulta, Praha 2010

The role of microRNAs in chronic lymphocytic leukemia

Martin Moravec

Mgr. Miroslav Hájek, PhD

Abstrakt

MikroRNA (miR, miRNA) je nově objevená skupina molekul (19-25 nukleotidů dlouhých), které regulují genovou expresi na post-transkripční úrovni buď zablokováním translace cílové mRNA, nebo degradací mRNA. Jako jedna ze součástí z mechanismu umlčování genů, miRNA se zapojují do buněčných procesů, jako například do procesu apoptózy, dělení a vývoje buněk a obrany proti virům. miRNA se v poslední době studují ve spojení k nemocem. Chronická lymfocytární leukémie (CLL) je jednou z nejběžnějších leukémií v západních zemích, která postihuje především starší lidi. V mojí práci se zaměřuji na vysvětlení funkce miRNA molekul a jejich vztah k chronické lymfocytární leukémii. Popisuji získáné poznatky o miRNA-15, miRNA-16, miRNA-143, miRNA-145 a miRNA-155 ve spojení k tomuto onemocnění. Na základě současných vědeckých článků diskutuji také potenciální roli miRNA-326 v CLL patogenezi.

Klíčová slova: **miRNA, mRNA, genová exprese, karcenogeneze, chronická lymfocytární leukémie**

Abstract

MicroRNAs (miRs, miRNAs) are recently discovered molecules (19-25 nucleotides long) that regulate gene expression at post-transcriptional level by either blocking protein synthesis or mRNA degradation. As a part of gene silencing mechanism, miRNAs are involved in cellular processes, such as apoptosis, cell proliferation, development and viral defence. miRNAs have been intensely studied in connection to disease pathogenesis. Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries affecting mostly elderly people. In my work I focus on explanation of miRNA functions and their contributions to chronic lymphocytic leukemia (CLL). I describe previously published data about miRNA-15, miRNA-16, miRNA-143, miRNA-145 and miRNA-155 in connection to this disease. Based on recent reports, I also discuss the potential role of miRNA-326 in CLL pathogenesis.

Key words: **miRNA, mRNA, gene expression, carcinogenesis, chronic lymphocytic leukemia**

Prohlašuji, že jsem tuto práci vypracoval samostatně za použití uvedené literatury a po konzultacích se svým školitelem.

.....

14.4. 2010 Martin Moravec

I would like to thank Dr. Miroslav Hájek for his mentorship, Dr. Tomáš Stopka and Pavel Burda for their valuable comments. Many thanks also go to Denmark to professors at the Aarhus University, namely Dr. Finn Skou Pedersen and Dr. Thomas Schmitt-John who introduced me to miRNA field that has truly attracted my attention and that I want to pursue in my future scientific career.

List of abbreviations

miRNA, miR	microRNA
mRNA	messenger RNA
TCL1	T cell lymphoma protein
PLAG1	pleomorphic adenoma gene 1
Elk-1	Ets-like protein 1
TSS	transcription start site
TFBS	transcription factor binding site
GTP	guanosin triphosphate
ATP	adenosine triphosphate
RISC	RNA-induced silencing complex
Ago	Argonaute protein
piRNA	piwi RNA
TF	transcription factor
RUNX	runt-related factors
ADAM	a disintegrin and metalloprotease
AP-1	activated protein 1
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
nt	nucleotide
bp	base pair
CLL	chronic lymphocytic leukemia
EST	expressed sequence tag
HPV	human papilloma virus
GDP	guanosin diphosphate
AID	activation-induced cytidine deaminase
SRE	serum response element
siRNA	short interfering RNA
ALL	acute lymphoblastic leukemia
TCF	ternary complex factor
PCR	polymerase chain reaction
ZAP-70	zeta-associated protein kinase
JAK	just another kinase
ORF	open reading frame
BIC	B cell integration cluster

Content

1. Introduction

RNA interference (RNAi) is a mechanism that silences gene expression at post-transcriptional level. Two major types of molecules participating in this process are small interfering RNAs (siRNAs) and recently discovered endogenous class of molecules - micro RNAs (miRNAs). miRNA discovery goes back to year 1993 when Lee and his colleagues indentified *lin-4* gene which did not code a protein, however lin-4 abolished normal embryo development in *C. elegans* (Lee et al., 1993). Since that, miRNAs and siRNAs have been getting much attention. In 2006, Andrew Fire and Craig Mello were awarded by Nobel Prize for the discovery of RNA interference. The mechanism by which these small molecules work is similar, in fact miRNA and siRNA regulate gene expression being a part of the same complex (see Section 4). In my work I describe miRNA function and its involvement in disease development.

2. Localization of miRNA genes

miRNAs are small molecules that control gene expression at post-transcriptional level by mRNA degradation or protein synthesis inhibition. According to the latest bioinformatic predictions there are roughly 1100 miRNAs encoded within human genome. To date only 721 of them have been identified (according to miRBase database www.sanger.co.uk/Rfam).

2.1. miRNA clusters

Interestingly, miRNA genes are often located in clusters and form polycistronic units (70% of all miRNAs). To this date, 51 clusters have been identified varying in number of miRNAs and their localization (with median 2,5 gene per cluster). The largest cluster consists of 8 genes and is located within chromosome 17. The shortest clusters consist of only two miRNAs. Interestingly, genes within the same cluster share homology at very high level suggesting that they control expression of the same or similar genes (Altuvia et al., 2005). On the contrary, a single miRNA can bind multiple genes due to incomplete complementarity. The clusters are located often at fragile sites within human genome (such as sites with high probability for chromatid exchange, translocation, deletion, amplification or integration sites for tumor-associated viruses like HPV). This indicates that deregulation of miRNA expression can be caused with high probability (Calin et al., 2004). A strong correlation was found between localization of specific miRNAs and localization of homeobox genes. The majority of them are located within homeobox genes or in their vicinity.

2.2. Intergenic and intronic miRNA genes and transcription

Many of the miRNA genes are located in intergenic regions (between two neighbouring protein-coding genes), but they were also found in intronic sequences. This fact has been confirmed by several recent studies suggesting that over 50% of all miRNA genes are situated in introns (Saini et al., 2008).

Intergenic miRNA genes are transcribed from their own promoters by RNA-polymerase II (Lee et al., 2004) or eventually by RNA-polymerase III (Borchert et al., 2006). The promoters in intergenic regions contain normal cellular promoter features such as TATA

boxes, transcription start sites (TSSs) and transcription factor binding sites (TFBSs). Also CpG methylation pattern plays important role in transcriptional regulation of miRNA genes. Recently a database called TransmiR was published summarizing the list of all transcription factors able to trigger miRNA genes transcription - to date, 82 proteins have been identified (Wang et al., 2009). Some of them have pleiotrophic effect. Interestingly, many proteins involved in development and cancer initiation serve as transcription factors for miRNA genes. Tumor suppressor p53 triggers miRNA-145 and miRNA-34 transcription. GATA-1 (transcription factor driving erythroid differentiation) induces expression of miRNA-144 and miRNA-451 (Dore et al., 2008).

Intronic miRNAs are mostly transcribed from the corresponding host promoters. Presently, it was suggested how intronic miRNA genes are processed. Bioinformatic approaches focusing on promoter features such as TSSs, TFBSs, CpG islands, ESTs and A/B box sequences (known RNA-polymerase III promoters) suggest that 35% of all intronic miRNAs include upstream promoters. Majority of these promoters are associated with RNA-polymerase II, while the rest is associated with RNA-polymerase III. The ability of these intronic genes to be transcribed independently on host promoter was also described. This fact actually brings another question – what is the character of the host promoter versus intergenic promoter regulation and whether RNA-polymerase II competes with RNA-polymerase III for promoter site (Monteys et al., 2010).

3. The biogenesis of miRNA

The transcription process starts in the nucleus where miRNA genes are transcribed mostly by RNA-polymerase II into large precursor, so called primary-miRNA (pri-miRNA).

3.1. The processing in nucleus

The pri-miRNA is several kilobases long transcript polyadenylated at 3' end and capped at 5' end. This precursor has an imperfectly pairing stem loop structure that is specifically recognised by RNase III enzyme Drosha which cleaves pri-miRNA into precurcor-miRNA (pre-miRNA). Pre-miRNAs are much shorter with usually 60-70 nucleotides per molecule (Lee et al., 2003).

3.2. The export into cytoplasm

As a result of Drosha cleavage, two nucleotide overhang at 3´ end of pre-mRNA is created (Okada et al., 2009). Two nucleotide overhang is recognised by Exportin-5 protein, which transports pre-miRNA from nucleus into cytosol in RAN-GTP dependent way. Exportin-5 protein is a double stranded RNA binding protein that transports not only all pre-miRNAs but also some tRNAs into cytosol. Importantly, Exportin-5 covers the two nucleotide overhang and protects pre-miRNA from ribonuclease digestion (Yi et al., 2003). In cytoplasm the pre-miRNA is dissociated from Exportin:RAN-GTP complex when GTP in RAN protein converts into GDP. This conformation change leads to a translocation of pre-miRNA into cytoplasm. Next, another member of RNase III family Dicer recognizes stem-loop- structured pre-miRNA and processes it into 19-25-nt long mature miRNA.

3.3. The processing in cytoplasm

Only one strand of miRNA (guide strand) is incorporated into RNA-induced silencing complex (RISC) which is a multiprotein complex regulating gene expression. The second strand (called passenger strand, miRNA*) is rapidly degraded. There has been extended discussion about how the leading strand is recognised and what factors and proteins determine this event. Some papers presume that Dicer and its helper protein R2D2 are main

players in selection process (Okamura et al., 2004; Ghildiyal et al., 2010). However, other research groups suggest that this process is performed by Argonaute proteins instead (Maniataki et al., 2005).

4. What is RISC complex?

RNA-induced silencing complex (RISC) consists of two components: a specific miRNA and specific proteins called Argonautes (Agos).

4.1. Argonaute proteins

Ago proteins are very important in functioning of the complex and their gene´s sequences are highly conserved. However, the number of Ago genes that are present in different species varies. For instance, *S. pombe* has just 1 gene encoding Ago protein, *D. megalomaster* has 5 genes, *A. thaliana* has 26 genes (which suggests that Ago proteins in plants underwent through extensive gene duplication). Ago proteins are divided into 3 paralogous groups, each having specific features (Liu et al., 2009).

- Argonaute-like proteins
- Piwi-like proteins
- Group 3 Argonautes

Interestingly, biochemical analysis of archaeal Ago proteins suggests that originally they functioned as RNAse H endonucleases that used DNA as a template. During evolution many Ago proteins either lost their DNA cleavage activity or specialized themselves on single-stranded RNA. Ago proteins consist of four domains that are important for their function (Fig. 2; Hutwagner et al., 2007).

The PAZ domain recognizes two nucleotide overhang at 3´end, which resulted from Drosha and Dicer cleavage. It is proposed that this feature is the main criterion for miRNA recognition, so other RNAs coming from other pathways will not enter miRNA pathway. Main function of PAZ domain is to bind ssRNA (Ma et al., 2004).

The PIWI domain is able to cleave ssRNA substrate- by using an RNase H-like motif (Asp-Asp-Asp/Glu/His/Lys) that they possess. The products of the cleavage also contain 3´-OH and 5´-P, which also reminds of RNase H processing.

The MID domain contains MC motif which has very similar motif to cap binding proteins such as translation initiation factor eIF4E. It is a very important feature that can play a significant role in regulation of translation (see Function of the RISC; Iwasaki et al., 2009).

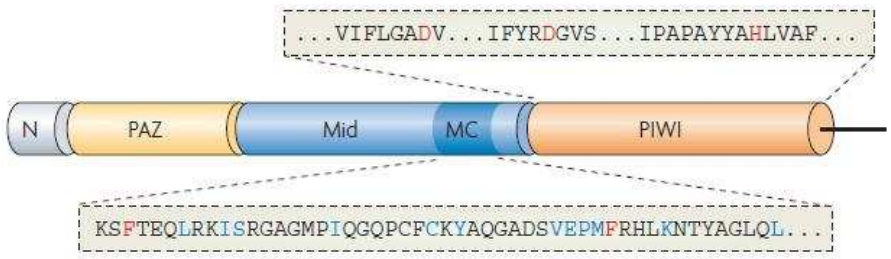

Fig. 1: Amino acid compostion of Argonaute proteins

Domain and amino acid sequence compositions of Ago proteins is shown. Their PAZ and PIWI domains seem to be well conserved across many species. (Hutwagner et al., 2007)

Human genome encodes 8 Argonaute proteins. Only 4 of them (namely Ago1, Ago2, Ago3 and Ago4) belong to Argonaute-like category, the rest 4 belong to Piwi-like proteins playing roles in piRNA pathways. piRNAs are recently discovered endogenous molecules with still unknown function. It is quite interesting to see that even though all 4 human Ago proteins in Argonaute-like category have PIWI domain, only Ago2 can cleave RNA. These findings are crucial for understanding of mechanisms how miRNAs regulate gene expression (Okamura et al., 2004).

4.2. Function of the RISC

As mentioned RISC is a ribonucleoprotein that contains specific leading strand of miRNA and various Ago proteins. The ability to control gene expression at post-transcriptional level by translation repression or mRNA cleavage is thought to be sequence-specific and comes from characteristics of miRNA and type of Ago proteins in the RISC. Every miRNA possesses an important sequence of 10 nucleotides called ”seed sequence” which is complementary to specific sequence in 3´ end UTR of the mRNA.

Complementarity between miRNA and mRNA in plants is high and therefore miRNA-mediated cleavage of mRNA is preferably observed (Bartel et al., 2004).

miRNAs can also mediate translation repression in case when complementarity between miRNA: mRNA is not perfect. It was proved that RISC can effectively block active site on mRNA so ribosomes can not associate with mRNA (Bartel et al., 2004). This could be seen mostly in vertebrates, humans including.

Recent evidence indicates that miRNA-mediated gene regulation can be caused by other mechanisms. For instance, by miRNA-mediated mRNA poly(A)deadenylation mechanism which affects mRNA stability (Fabian et al., 2009). Also MID domain of Ago proteins can be important in protein translation initiation by competing with eIF4E for cap structure at 5´ end of mRNA (Iwasaki et al., 2009).

Interestingly, RISC can also function as a translation activator by recognising specific AU sequence at 5´ end of mRNA (Henke et al., 2008).

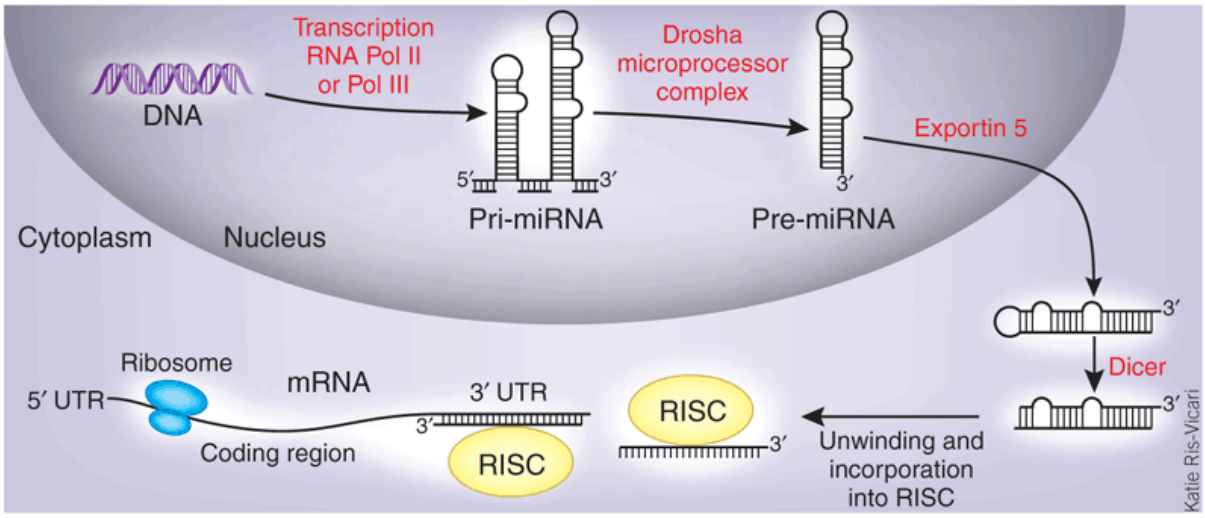

Fig. 2: miRNA biogenesis with all its important steps

miRNA genes are transcribed by RNA-polymerase II or RNA-polymerase III. Then pri-miRNAs are processed and exported into cytoplasm, where mature forms of miRNA are incorporated into RISC complex leading to targeting of a mRNA processing. (http://www.nature.com/nbt/journal/v26/n4/images)

5. Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is a type of leukemia characterized by abnormal accumulation of mature B lymphocytes in blood, bone marrow, spleen and lymph nodes that are incompetent in their function. CLL accounts about 30 % of all known leukemias. This number classifies CLL to one of the most common leukemia in Western countries affecting mostly elderly people. Most patients diagnosed by CLL are 70 years and older, nearly no patients are diagnosed at young age. Even though a lot is known about CLL pathogenesis, understanding of the disease initiation is still poor (Chen et al., 2008).

B cells affected by CLL express B lymphoid surface antigens CD5+, CD19+, CD20+ and CD23+. Most of these cells remain rather in resting Go phase than in M phase. It has been estimated that only 27 % of all B cells remain proliferative, the rest of the population remains in resting mode. Usually, healthy B cells can survive a few days, B-CLL cells can circulate in blood for several months. High survival rate of B-CLL cells suggests that a problem in apoptosis initiation could occur. Next, it was found that B-CLL cells highly express **Bcl-2** as a consequence of translocation of bcl-2 gene into heavy chain region of immunoglobulins, which places this gene under strong enhancer control. Bcl-2 protein belongs to Bcl-2 family and serves as anti-apoptotic factor (Cerroni et al., 1994).

5.1. Genetics and clinical outcome

FISH technique (fluorescence in situ hybridization) reveals differences in chromosomal features between healthy donors and CLL patients:

Most of all CLL patients have deleted 30 kb region on chromosome 13. There were also found deletions or mutations in p53 gene on chromosome 17 in 15% of CLL patients. This particular defect marks more aggressive disease. Deletion of ATM gene on chromosome 11q22 was also observed. Other abnormalities, such as deletions on chromosome 2, 14, 17 and 20 can be also important in CLL pathology (Trojani et al., 2010).

CLL varies in clinical outcomes based on other prognostic markers. Mutational status of the immunoglobulin heavy chain is a marker of non-aggressive CLL. On the other hand, CLL patients with unmutated immunoglobulin heavy chain have shorter median survival. Also presence or absence of ZAP-70 kinase distinguishes different CLL prognosis (Trojani et al., 2010).

6. miRNAs and CLL pathogenesis

miRNAs have been found to be aberrantly expressed in several human diseases – including CLL. In cancer, some miRNAs can act as oncogenes (oncomiRs) or tumor suppressors. The oncomiR regulates expression of cellular tumor suppressor protein, therefore its upregulation leads to cancerigenesis. In contrast, downregulation of miRNAs regulating expression of cellular oncogenes can also induce cancer.

6.1. The miRNA-15a and miRNA-16-1 cluster

As already mentioned, one of the chromosomal marks of CLL is a 30 kb deleted region on chromosome 13 (in 50 % of all patients). In addition, this deletion was found in other cancers suggesting its important role in cancerigenesis in general. Therefore there has been an effort to identify genes within this region. Calin and his colleagues managed to identify a cluster of two miRNA genes – miRNA-15a and miRNA-16-1. In order to clarify their findings, they used Northern blot analysis of to that date known miRNAs. And they compared them with CLL samples, prostate cancer samples and healthy CD5+ samples. A remarkable downregulation in miRNA-15a and miRNA-16-1 was found in 68% CLL samples and in 90% of prostate cancer samples. It was confirmed that this downregulation was a result of deletion on chromosome 13. However, miRNA-15a and miRNA-16-1 did not show correlation with predicted target genes (Calin et al., 2004).

6.1.1. What genes are targeted by miRNA-15a and miRNA-16-1?

Better bioinformatic tools were able to predict more potential targets of miRNA-15a and miRNA-16-1. ***BCL-2*** gene was predicted to be a primary target of miRNA-15a and miRNA-16-1 (Cimmino et al., 2005).

Further experiments on specific cell lines with expression vectors carrying miRNA-15a and miRNA-16-1 sequences, antisense nucleotides and analysis methods strongly confirm that levels of bcl-2 protein in the cell correlate with miRNA-15a and miRNA-16-1 concentration. Also higher expression of these miRNAs activated intrinsic APAF1-caspase9-

PARP-mediated apoptosis pathway in CLL cells (Cimmino et al., 2005). Recently, 5 other targets of miRNA-15a and miRNA-16-1 in CLL have been suggested to contribute to leukemiagenesis- ***BAZ2A, RNF41, RASSF5, MKK3,*** and ***LRIG1*** (Hanlon et al., 2009).

The ***BAZ2A*** *(*bromodomain adjacent to zinc finger domain 2A, Tip5) gene is a member of the bromodomain family promoting its function in chromatin remodeling complexes. Interestingly, deregulation of BAZ2A protein in pre-B cells triggered acute lymphoblastic leukemia (ALL).

RNF41 (ring finger protein 41) protein is an ubiquitin ligase that is thought to be involved in etiology of hematological malignancies and which seems to be important in cell lineage differentiation.

RASSF5 (Ras association domain family member 5) is a member of the RAS family and plays important role in signal transduction pathways – very important in cell cycle control.

MKK3 (mitogen-activated protein kinase kinase 3, MEK3) serves as an activator in p38 MAPK pathway. This pathway was investigated to be activated in B-CLL cells but not in normal peripheral B cells. Also ***LRIG1*** (leucine-rich repeats and immunoglobulin-like domains 1, LIG1) was found downregulated in CLL cells, LRIG1 is proposed as tumor suppressor gene that regulates signaling in the cell by ubiquitin ligases recruitment. However, its function remains unclear (Hanlon et al., 2009).

Although there have been identified several genes and suggested their role in cancerigenesis, it is difficult to predict whether their deregulation is a hallmark of cancerigenesis or whether their deregulation is a consequence of other factors in CLL.

6.2. miRNA-143 and miRNA-145 cluster

miRNA-143 and miRNA-145 are localized within a 1,8 kb distance from each other and they are classified as members of the same cluster. miRNA-143 and miRNA-145 are associated not only with CLL, but also with other cancers, such as prostate cancer, colon cancer, bladder cancer and so on. In all these diseases, miRNA-143 and miRNA-145 were found to be significantly downregulated compared to cells from healthy donor (Akao et al., 2007). This suggests that function of these miRNAs is not restricted only to hematological malignancies but to cancer in general.

The cluster of miRNA-143 and miRNA-145 genes is located on chromosome 5, interestingly there are no observations that downregulation of these miRNAs was caused by loss or deletion of the 1,8 kb region suggesting that this loci is transcriptionally inactive in cancer.

6.2.1. Function of the cluster

miRNA-143 and miRNA-145 expression profile was clarified in 22 B-cell malignancies by RT-PCR method. Among those B-cell malignancies, there were chronic lymphocytic leukemia samples and B-cell lymphoma samples. In all diseases, miRNA-143 and miRNA-145 were low-expressed compared to controls (CD19+ B cells). Validation of miRNA-15a expression in the CLL samples showed downregulation in approximately 50 % of samples.

For further experiments Raji B-cell line was selected. This cell line has very low expression of miRNA-143 and miRNA-145. As expected, transfection of miRNA-143 and miRNA-145 caused growth inhibition, which appeared to be dose-dependent, suggesting that miRNA-143 and miRNA-145 are repressors of cell growth (Akao et al., 2007).

Potentially miRNA-143 can regulate **mitogen-activated protein kinase (ERK5) –** the concentration of miRNA-143 correlated with ERK5 expression in the Raji cell line. ERK5 is a MAP kinase that activates **myelocytomatosis oncogene (MYC)** which is one of downstream components in signal transduction process. Notably, the levels of ERK5 mRNA were found unchanged in experiments which suggests that miRNAs abolished protein synthesis (Akao et al., 2007).

Evidence that also miRNA-145 influences active MYC concentration in the cells comes from another group (Sachdeva et al., 2009). miRNA-145 directly base pairs with 3´ UTR of MYC mRNA. MYC (c-myc) is a transcription factor that regulates expression of many genes involved in cellular processes and its deregulation has been observed in many cancers. For instance, in 30 – 50% patients with breast cancer have MYC overexpression (McNeil et al., 2006). MYC is one of the proteins that have ability to drive cells into S phase through activating expression of CDK4 (cyclin-dependent kinase 4) and eIF4E (eukaryotic translation initiaton protein) – a very important hallmark of cancer initiation (Sachdeva et al., 2009).

6.2.2. p53- miRNA-145 loop

Recently it was found that miRNA-145 downregulates MYC through imperfect base pairing with 3´ UTR of **MYC** mRNA. In addition to that, further experiments discovered that miRNA-145 expression in cells is very precisely controlled by p53 (Fig. 4). Since p53 is critical in cell cycle inhibition, its mutation can be significant for cell cycle control. In fact, there could be more genes that correlate with miRNA-145 expression. As miRNA-145 was found downregulated in many cancers, it would be interesting to investigate whether it happened through p53 inactivation or whether other proteins are involved in this process.

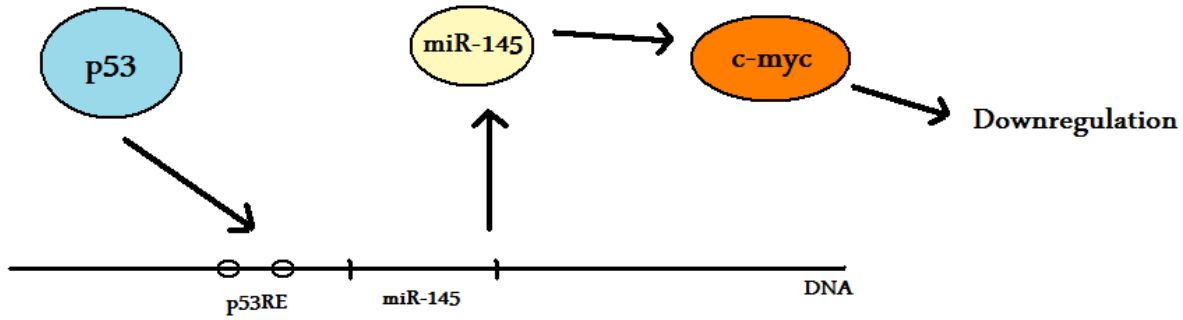

Fig. 4: Possible still not fully proved mechanism of miRNA-145 role in cancer

P53 binds to p53 response elements (p53REs) and directs transcription of miRNA-145 gene. Product of miRNA-145 gene in turn influences translation of c-myc.

Recent findings from colon cancer cells suggest that miRNA-145 can contribute to cancer progression through ***YES*** (Yamaguchi sarcoma viral oncogene homolog 1) and ***STAT1*** (signal transducer and activator of transcription 1) downregulations (Gregersen et al., 2010). YES is a tyrosine kinase that belongs to Src kinase family and its upregulation affects cell motility and growth. STAT1 is a transcription factor that is located downstream of JAK. STAT1 can work as pro-apoptotic stimulator, but also its deregulation can have pro-survival function in some cancers. Experiments with luciferase assay suggested that miRNA-145 directly binds to STAT1 and YES mRNAs. Interestingly, STAT1 does not have any match with seed sequence of miRNA-145 with 3´ UTR, it has 7 mer alignment in coding region of mRNA. 3´ UTR of YES does possess a specific sequence pairing with miRNA-145 (Gregersen et al., 2010).

6.3. miRNA-155

While miRNA-143 and miRNA-145 seem to be downregulated in CLL and in other cancerous cells, miRNA-155 has been found upregulated in wide array of cancer. Especially, in lymphomas and myeloid leukemias miRNA-155 expression is very high - 15- 25-fold higher than concentration in healthy B and T cells. miRNA-155 comes from precursor BIC (B-cell integration cluster). The BIC is polyadenylated RNA with length of 1.7-kb which possesses 3 ORFs, however it is thought that none of them encodes protein (Eis et al., 2005).

The BIC/miRNA-155 gene is located on chromosome 21 and there is a classical TATA box 24 nt upstream from the start site. In order to understand how high levels of miRNA-155 concentration in unhealthy cells can occur, to focus on transcription factors is essential. The identifying of transcription factors seems to be even more important with no deletion in BIC/miRNA-155 gene observations. The comparison of homology sequences between mouse and human reveals that there is AP-1 transcription factor binding site upstream of TATA box and c-Ets binding site downstream of TATA box (Fig. 5). Nevertheless, there was also found NF-kappaB site upstream of TATA box but NF-kappaB is not positionally conserved (Wang et al., 2008).

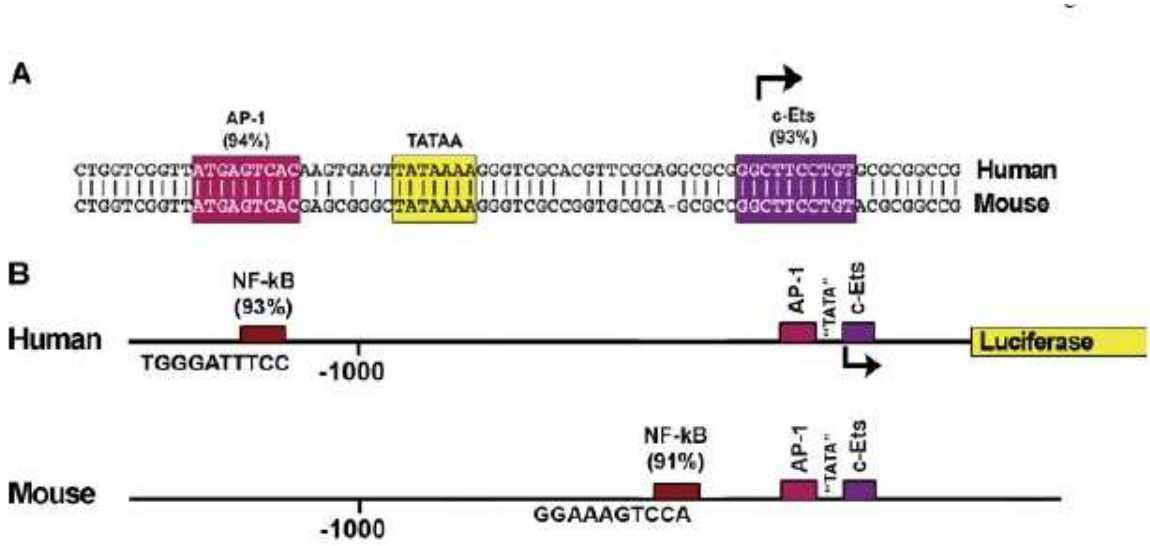

Fig. 5: Localization of transcription factor sites in the vicinity of miRNA-155 promoter

A) Localization of transcription factors binding sites AP-1 and c-Ets in neighborhood of miRNA-155 promoter.

B) Comparison between mice and human NF- kappaB sites reveals that NF- kappaB localization is in further distance in humans than in mice. (Wang et al., 2008)

It was discovered that ***transcription factors AP-1*** (namely JunB and FosB) binding to AP-1 site are very successful in transcription initiation. c-Ets and NF-kappaB seem not to be that important in BIC/miRNA-155 gene transcription. Even though AP-1 transcription factors seem to be extremely important for miRNA-155 expression, there could be other factors which can either contribute to AP-1 function or take over the role. Very high concentration of miRNA-155 in CLL cells can be results from high BCR stimulation, processing signals through ERK and JNK pathways to cell nucleus and to BIC/miRNA-155 transcription (Wang et al., 2008).

Recently, it has been suggested that concentration of miRNA-155 goes up after toll-like receptor stimulation by lipopolysacharide (Tili et al., 2007).

6.3.1. Function of miRNA-155

Recently two independent studies clarify that ***inositol phosphatase SHIP1*** is a primary target of miRNA-155 (Pedersen et al., 2009, O´Connell et al., 2009). There was found a 8 mer miRNA-155 pairing sequence at 3´ UTR of SHIP1 gene. SHIP1 negatively regulates phosphatidylinositol 3-kinase (PI3K) signaling that controls fate determination in B cells (Srinivasan et al., 2009). Notable, it was only the sequence of miRNA-155 that was found conserved at 3´ UTR of SHIP1 among other species. Further experiments with mutants in reporter systems revealed that concentration of miRNA-155 correlates with SHIP1 concentration in these cells and SHIP1 total knockdown by miRNA-155 or specific siRNA influences differentiation of cells in bone marrow – SHIP1 knockdown leads to a decrease in lymphocytes numbers and to an increase of myeloid cells. Therefore miRNA-155 could be a contributing factor in human myeloid leukemias in which miRNA-155 has been found upregulated (O´Connell et al., 2009).

Interestingly, major lineage-determining **transcription factor PU.1** has been suggested to be a direct target of miRNA-155 due to its target sequence at 3´ UTR of PU.1 (Vigorito et al., 2007; Eis et al., 2005). PU.1 directs myeloid transcriptional program giving rise the population of granulocytes and macrophages. However, levels of PU.1 below certain threshold block hematopoietic differentiation (Burda et al., 2009). Therefore, PU.1 downregulation following by miRNA-155 upregulation may lead to oncogenesis.

6.4. Other miRNAs with oncogenic or tumor suppressive functions in CLL

6.4.1. miRNA signatures in CLL in comparison to healthy B cells

It is not easy to predict what is the role of miRNAs in CLL etiology. One possible approach is to make miRNA profiling and compare expression profiles between CLL cells and healthy cells (Calin et al., 2004). There is a general idea today that significant downregulation or upregulation of one (or more) specific miRNA can be crucial in disease progression by targeting specific tumor suppressors, oncogenes and cell-cycle genes. Therefore miRNA profiling can give us hint of function of miRNA in the cell. One of the latest studies suggests that many miRNAs are downregulated in CLL (compared to CD 19+ healthy donors). Namely, ***miRNA-181a, miRNA-181b, miRNA-326, miRNA-107, miRNA-424, miRNA-125a and miRNA-126 were found significantly downregulated in CLL*** (Pallash et al., 2009). This study also shows that these downregulations are not a result of deletions, but epigenetic inactivation by promoter methylation.

6.4.2. PLAG1 targeted by miRNA-181a+b

In order to search potential targets of these miRNAs applying bioinformatics is crucial. Bioinformatics approaches suggested that **pleomorphic adenoma gene 1 (PLAG1)** is a good candidate gene, because PLAG1 3´ UTR carries many targeting sites (Fig. 6). In order to clarify these predictions experiments applying luciferase assays, expression vectors and Western blot analysis were applied. As expected, experiments indicate that miRNA-181a, miRNA-141, miRNA-424, and miRNA-107 bind to 3´ UTR of PLAG1. Because of localization of two conserved miRNA-181a+b binding sites and one poorly conserved site within 3´ UTR PLAG1 mRNA, the focus on function of miRNA-181a+b in CLL cells was emerged (Fig. 6). Correlation between miRNA-181a+b and PLAG1 protein level was observed in CLL cells in which PLAG1 expression was higher compared to healthy controls (Pallash et al., 2009).

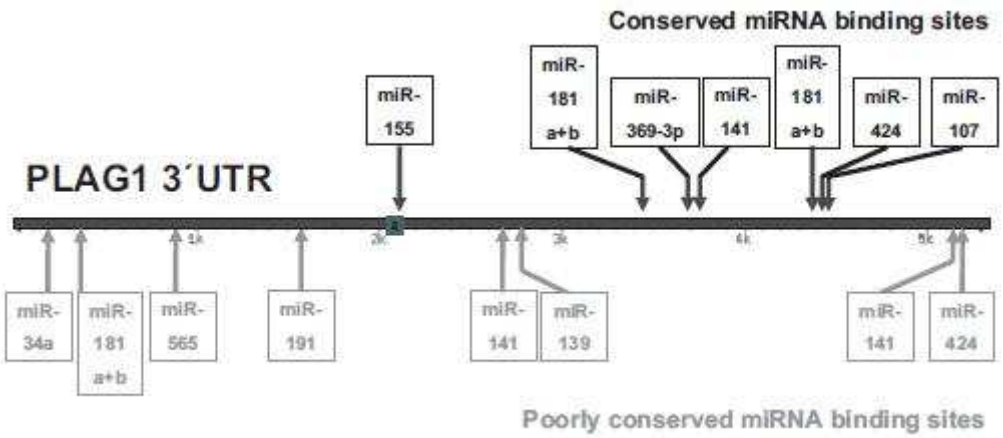

Fig. 6: 3´UTR of PLAG1 and miRNAs

Region of 3´ UTR of PLAG1 mRNA has many conserved binding sites for miRNAs. Many of those miRNAs were found downregulated in CLL. There are also a lot of poorly conserved binding sites suggesting that so many miRNAs can affect PLAG1 expression. (Pallash et al., 2009)

These findings led to a question whether PLAG1 can influence pathogenesis in CLL. PLAG1 is a zinc-finger transcriptional factor previously described in gland adenomas, breast cancer and acute myeloid leukemia as oncogenic activator when overexpressed (Dyck et al., 2007). Therefore, implication of PLAG1 in CLL could be an important player in disease progression. However, there is only one study about implication of PLAG1 in CLL disease (Pallash et al., 2009).

It seems that miRNA-181a+b are important in CLL not only by targeting PLAG1, but according to other recent studies miRNA-181b also controls levels of **TCL1 (T-cell lymphoma 1A)** protein (Pekarsky et al., 2006). TCL1 is an important oncogene in CLL that correlates with higher expression of pro-survival factors in many cell-lines, B and T cell lines including. However, miRNA-181 is one of the miRNAs expressed specifically in hematopoietic cells in general (Merkerova et al., 2008). It has been suggested that TCL1 works as ***co-activator of Akt pathway***. To this date, there is evidence available about Akt regulating survival and proliferation rate (Laine et al., 2000).

6.4.3. Potential function of miRNA-326 in CLL

According to recently published study several miRNAs were found downregulated in CLL (Pallash et al., 2009). One of them, ***miRNA-326***, was nearly 15-fold less expressed in CLL than in healthy B cell samples. This remarkable fold change in miRNA expression led me to ask question what is the function of miRNA-326 in CLL cells. Next, I tried to presume potential function of miRNA-326 in B cells and its potential contribution to cancerigenesis. To this date (March 2010), there are only 3 papers published about miRNA-326. In one of them, miRNA-326 regulates Th-17 lymphocyte differentiation by targeting Ets-1 transcription factor (Du et al. 2009). Second and third publications refer to an implication of miRNA-326 in neural cancer cells, in which miRNA-326 inhibits one component of Notch pathway (protein Smoothened) and therefore reduces viability, proliferation and invasiveness of glioblastoma cells (Ferretti et al., 2008; Kefas et al., 2009).

I used bioinformatic programs TargetScan (http://www.targetscan.org) and MiRBase (http://www.mirbase.org) for target predictions. I selected these programs because their algorithms use latest observations from miRNA field. I focused only on genes that can play role in cancerigenesis, such as oncogenes, tumor suppressor genes, cell cycle genes, signal transduction genes, transcription factors. Any genes out of this criterion were not taken into my "study". For illustration, I selected 13 potential targets from TargetScan and 26 from MiRBase (Table 1). In addition to different targets suggested by each algorithm, two matches were obtained – zinc finger protein 322A (ZNF322A) and **ELK1** protein, both of them being transcription factors.

A list of putative targets of miR-326 with oncogenic potential			
Probability	Database	Gene ID	Name
1.	**targetscan**	**ZNF322A**	**zinc finger protein 322A**
2.	targetscan	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
3.	targetscan	RAP2A	RAP2A, member of RAS oncogene family
4.	targetscan	WDR32	WD repeat domain 32
5.	**targetscan**	**ELK1**	**ELK1, member of ETS oncogene family**
6.	targetscan	PIP4K2C	phosphatidylinositol-5-phosphate 4-kinase, type II, gamma
7.	targetscan	PPP2R5B	protein phosphatase 2, regulatory subunit B', beta isoform
8.	targetscan	ZZEF1	zinc finger, ZZ-type with EF-hand domain 1
9.	targetscan	MAPK4	mitogen-activated protein kinase 4
10.	targetscan	ZCCHC14	zinc finger, CCHC domain containing 14
11.	targetscan	RIT1	Ras-like without CAAX 1
12.	targetscan	RUNX3	runt-related transcription factor 3

13.	targetscan	LDOC1	leucine zipper, down-regulated in cancer 1
1.	miRDB	RALGPS2	Ral GEF with PH domain and SH3 binding motif 2
2.	miRDB	NLK	nemo-like kinase
3.	**miRDB**	**ZNF322A**	**zinc finger protein 322A**
4.	miRDB	CRTC3	CREB regulated transcription coactivator 3
5.	miRDB	SMAD3	SMAD family member 3
6.	miRDB	LRRC15	leucine rich repeat containing 15
7.	miRDB	LONRF2	LON peptidase N-terminal domain and ring finger 2
8.	miRDB	MOBKL2C	MOB1, Mps One Binder kinase activator-like 2C (yeast)
9.	miRDB	MRAS	muscle RAS oncogene homolog
10.	miRDB	PPP1R3F	protein phosphatase 1, regulatory (inhibitor) subunit 3F
11.	**miRDB**	**ELK1**	**ELK1, member of ETS oncogene family**
12.	miRDB	HIPK2	homeodomain interacting protein kinase 2
13.	miRDB	BHLHB5	basic helix-loop-helix domain containing, class B, 5
14.	miRDB	ADAMTS18	ADAM metallopeptidase with thrombospondin type 1 motif, 18
15.	miRDB	PRAMEF8	PRAME family member 8
16.	miRDB	RAP2A	RAP2A, member of RAS oncogene family
17.	miRDB	ZC3H12B	zinc finger CCCH-type containing 12B
18.	miRDB	PKIA	protein kinase (cAMP-dependent, catalytic) inhibitor alpha
19.	miRDB	SH3PXD2A	SH3 and PX domains 2A
20.	miRDB	ARPP-21	cyclic AMP-regulated phosphoprotein, 21 kD
21.	miRDB	FGD3	FYVE, RhoGEF and PH domain containing 3
22.	miRDB	CCNJL	cyclin J-like
23.	miRDB	LAT2	linker for activation of T cells family, member 2
24.	miRDB	FBXL18	F-box and leucine-rich repeat protein 18
25.	miRDB	STK35	serine/threonine kinase 35
26.	miRDB	RHOT1	ras homolog gene family, member T1

Table 1: Potential miRNA-326 cancer-related targets

I next searched for function of some candidates in Table 1 and their implications in cancer.

6.4.3.1. Potential function of Elk- 1 in CLL

Elk-1 (Ets-like 1) protein is a member of ***ternary complex factors*** family ***(TCFs).*** These proteins are important in transcription of genes upon extra-cellular signals and/or stress-mediated signals. Surprisingly, another member of this family is PU.1 that is lineage-determining transcription factor. Proteins of the TCF subfamily form a ternary complex by binding to the serum response element in the promoter of the ***FOS*** and ***EGR1*** proto-oncogenes. The protein encoded by *FOS* gene form a dimer with Jun proteins. These

complexes are called as activated-protein 1 complexes (AP-1) and are nuclear target for the ***ras-raf-MAPK*** signaling cascade, main pathway of cell proliferation (Fig.7; Yang et al., 2003).

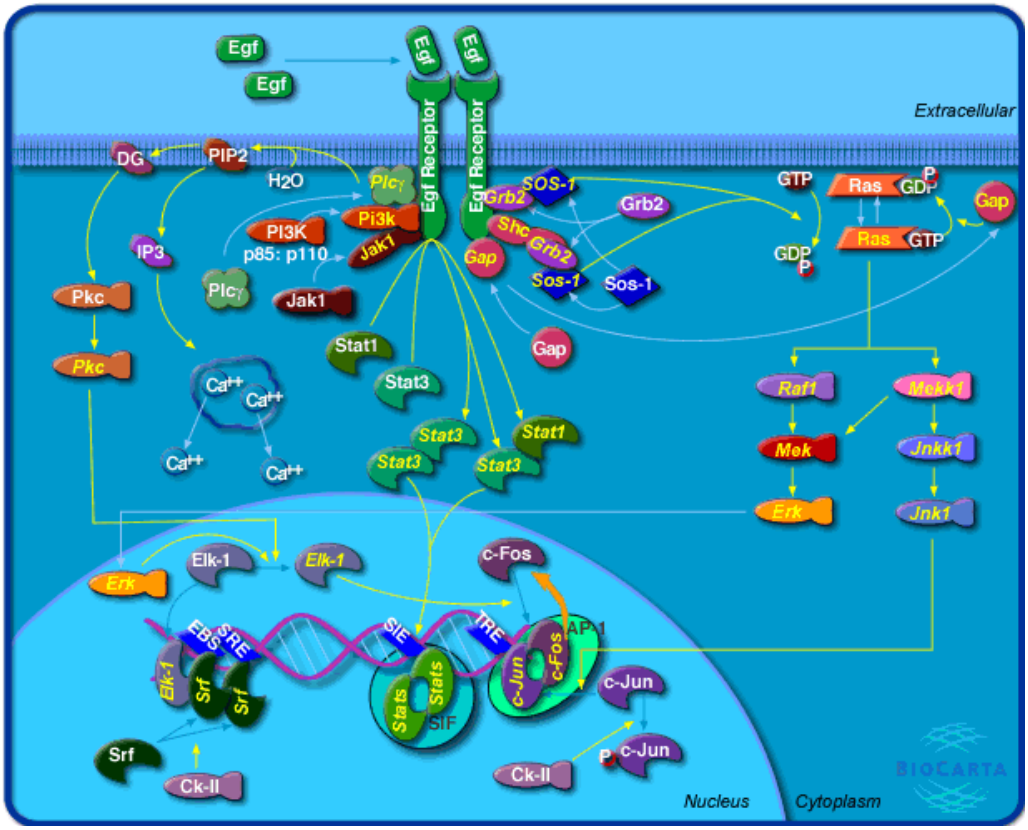

Fig. 7: A signaling cascade inside the cell

The Elk-1 is activated by Erk which phosphorylates serine at position 383 on Elk-1. Activated Elk-1 binds to SRE elements on DNA and serves as transcription factor positively regulating transcription of many genes. (www.kugi.kribb.re.kr)

6.4.3.1.1. Elk- 1 and AP-1

I searched for potential involvement of Elk-1 in tumorigenesis. Elk-1 positively regulates transcription not only of FOS protein but also of ERG1 protein, which is an oncogenic transcription activator (Reddy et al., 1991). Levels of FOS protein, being part of AP-1 complex, can determine the expression of other genes and miRNAs in cells such as miRNA-155 which is overexpressed in CLL (Section miRNA-155). Interestingly, there is also a study suggesting that upregulation of Elk-1 can be important in cancer development (Chen et al., 2006).

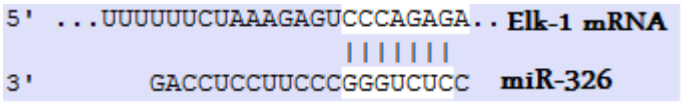

Fig. 8: Alignment between Elk-1 mRNA and miRNA-326

Base pairing between seed sequence of miRNA-326 and 3´ UTR of Elk-1 mRNA between nucleotides 1240-1246 suggests that this protein can be affected by miRNA-326 in cells.

6.4.3.2. RUNX as potential target of miRNA-326?

Although I have tried to explain how Elk-1 could contribute to leukemiagenesis the Table 1 contains many more cancer-related genes that can be potentially targeted by miRNA-326 with high probability.

For instance, ***RUNX proteins*** (Runt-related transcription factors) have been shown upregulated in gastric cancer (Hsu et al., 2009). And knockdown of RUNX3 in B cells induced RUNX1 expression and inhibition of cell proliferation suggesting it may play role in B cell pathology process (Whiteman et al., 2006).

7. Discussion

I have summarized results of others suggesting that miRNAs can contribute to cancerigenesis by many mechanisms. There is a general idea that cancers can be caused by deregulation of one or more specific transcription factors (miRNAs including) that disrupt balance within cell.

For illustration, I focused on potential consequences of miRNA-326 downregulationin CLL. miRNA-326 gene is located in intron of protein beta-arrestin on chromosome 11 and can potentially target Elk-1. Bioinformatic algorithms suggest that miRNA-326 can target many cancer-related genes with high probability (Table 1).

Although the miRNA field is a very new area of molecular biology which is getting more and more attention - it has been proposed its implication in medicine. For instance, miRNA profiling could serve as important biomarkers in disease development. Or more importantly as prognostic marker which can distinguish different types of diseases, such as cancer (Garzon et al., 2008). For instance, several studies have been published about differential miRNA expression in rare forms of leukemia (Eis et al., 2005).

To date, miRNA-155 upregulation (Eis et al., 2005), miRNA-143 and miRNA-145 downregulation seem to be reliable biomarkers in CLL (Akao et al., 2007). Downregulation of miRNA-15a and miRNA-16-1 in CLL has become recently very controversial and it is being greatly discussed (Sachdeva et al., 2008). Therefore, their usability as biomarkers is not fully confirmed.

miRNA profiles are tissue specific and thus miRNA signature can differ from cell to another (Fig. 9; Merkerova et al., 2008), which brings another challenge into biomarker implication. Interestingly, findings about involvement of miRNAs in cellular pathways can predict more efficient treatment.

RNAi-based therapeutics seem to have a great potential in the future. I already found two RNAi-based drugs in clinical trials. One is based on let-7 miRNA, which targets specifically RAS oncogene in lung cancer (Johnson et al., 2005). The other one is miRNA-122-based drug against hepatitis C virus. I suppose that results obtained from these tests will highlight a way for future therapeutic design.

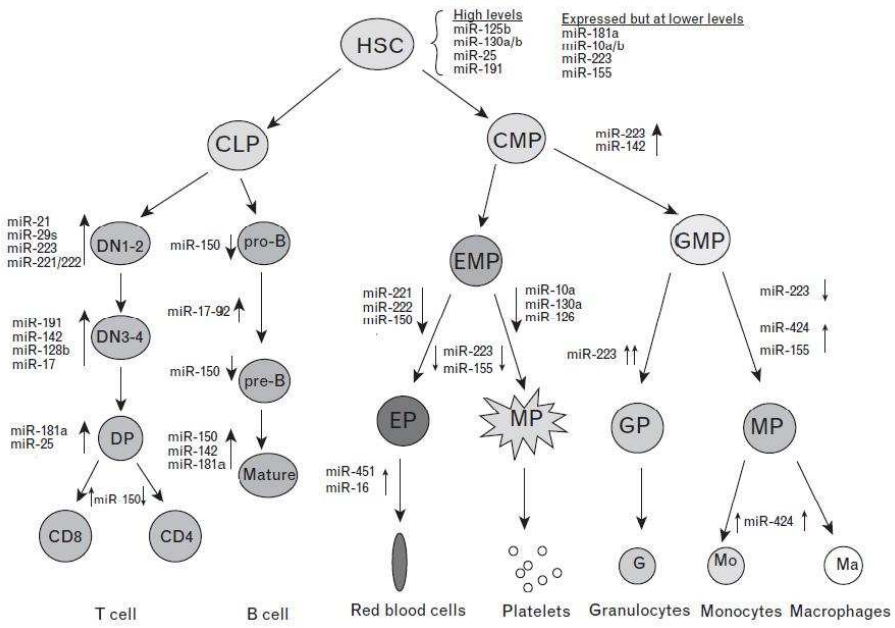

Fig. 9: Tissue specific miRNA signatures in hematology cells

miRNA profiles differ during development of the cell. CLP= common lymphoid progenitor, CMP= common myeloid progenitor, HSC= hematopoietic stem cells, GMP= granulocyte-monocyte precursor, DP= double positive cells (CD4+CD8+ T cells), DN= double negative T cell precursors, EP= erythrocyte precursor, MP= megakaryocyte precursor. (Garzon et al., 2008)

miRNAs put the area of gene regulation to another level. As one miRNA can regulate function of several mRNAs (up to 200 mRNAs) and thus tune various biological pathways. In fact, we are talking about network that controls another network suggesting how complex the system in cells could be. Interestingly, it gives cells more options how to synchronize processes in deficiency of one particular gene (one can imagine that some pathways can take over the function of old or not-working pathway). However, it can also higher the probability of disease development when some crucial miRNA is mutated.

Supplement

As miRNA field is very new, special terminology had to be developed. Every miRNA can be found in miRNA registry (http://www.sanger.ac.uk/software/rfam/mirna) under its number and name. Most miRNAs have their names according to the chronological order of their discovery. The rest of miRNAs still have their original name, e.g. lin-4.

If there is a distinct letter placed in the end of miRNA designation, e.g. miRNA-181a and miRNA-181b, it represents a sequence difference (usually 1 nt or 2 nt) between these 2 molecules of miRNAs. For instance, miRNA-181a and miRNA-181b differ from each other only in one or two nucleotides. If the identical mature miRNA sequences originate from different genomic loci in the same organism (e.g. different chromosome) then the mature forms are distinguished with numbers behind the name. For instance, miRNA-6-1 and miRNA-6-2 have the same sequence but they are not encoded in the same genomic loci (Griffiths-Jones, 2003).

8. List of references

1. Akao Y, Nakagawa Y, Kitade Y, et al. Downregulation of microRNAs-143 and -145 in B-cell malignancies. Cancer Sci 2007; 7: 1-7

2. Altuvia Y, Landgraf P, Lithwick, et al. Clustering and conservation patterns of human microRNAs. Nucleic acids research 2005; 33(8): 2697-2706

3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism and function. Cell 2004; 116(2):281-97

4. Borchert GM, Lanier W, Davidson BL, et al. RNA polymerase III transcribes human microRNAs. Nat struct mol boil 2006; 13: 1097-1101

5. Burda P, Curik N, Stopka T, et al. PU.1 activation relieves GATA-1-mediated repression of Cebpa and Cbfb during leukemia differentiation. Mol Cancer Res 2009; 7(10): 1693- 1703

6. Calin GA, Croce CM, Dumitru CD, et al. Frequent deletions and down-regulation of microRNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. PNAS 2002; 99(24): 15524-15529

7. Calin GA, Liu CG, Sevignani C, et al. MicroRNA profiling revelas distinct signatures in B cell chronic lymphocytic leukemias. PNAS 2004; 101(32): 11755-11760

8. Calin GA, Sevignani C, Croce CM, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. PNAS 2004; 101(9): 2999-3004

9. Cerroni L, Volkenandt M, Rieger E, et al. Bcl-2 protein expression and correlation with the interchromosomal 14; 18 translocation in cutaneous lymphomas and pseudolymphomas. J invest Dermatol 1994; 102(2): 231-235

10. Chen AG, Yu ZC, Yu XY, et al. Overexpression of Ets-like protein 1 in human esophageal squamous cell carcinoma. WJG Press 2006; 12(48): 7859-7863

11. Chen J, McMillan NAJ. Molecular basis of pathogenesis, prognosis and therapy in chronic lymphocytic leukaemia. Cancer biology and therapy 2008; 7(2): 174-179

12. Cimmino A, Calin GA, Croce CM, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. PNAS 2005; 102(39): 13944-13949

13. Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003; 425(6956):415-9

14. Dore LC, Amigo JD, Santos CO, et al. A GATA-1-regulated microRNA locus essential for erythropoiesis. PNAS 2008; 105(9): 3333-3338

15. Du C, Liu C, Kang J, et al. MicroRNA miR-326 regulates Th-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nature immunology 2009; 10(12): 1252-1260

16. Dyck FV, Declercq J, Braem CV, et al. PLAG1, the prototype of the PLAG gene family: Versatility in tumour development. International J of Onco 2007; 30: 765-774

17. Eis PS, Tam W, Chadburn A, et al. Accumulation of miR-155 and BIC RNA in human B cell lymphoma. PNAS 2005; 102(10): 3627-3632

18. Fabian MR, Mathonnet G, Sundermeier T, et al. Mammalian miRNA RISC recruits CAF1 and PABP to affect PABP-dependent deanylation. Mol Cell 2009; 35(6): 868-880

19. Feretti E, Smaele DE, Miele E, et al. Concerted microRNA control of Hedgehog signaling in cerebellar neuronal progenitor and tumour cells. The EMBO Journal 2008; 27: 2616–2627

20. Garzon R, Croce CM. MicroRNAs in normal and malignant hematopoiesis. Curr opin hematol 2008; 15: 352-358

21. Ghildial M, Xu J, Seitz H, et al. Sorting of Drosophila small silencing RNAs partitions microRNA* strands into the RNA interference pathway. RNA 2010; 16(1): 43-56

22. Gregersen LH, Jacobsen AB, Frankel LB, et al. MicroRNA-145 targets YES and STAT1 in colon cancer cells. PLoS 2010; 5(1): 1-10

23. Griffiths-Jones S. The microRNA registry. Nucleic acids research 2004; 32: D109-D111

24. Hanlon K, Rudin CE, Harries LW. Investigating the targets of MIR-15a and MIR-16-1 in patients with chronic lymphocytic leukemia. PLoS 2009; 4(9): 1-7

25. Hein S, Mahner S, Kanowski C, et al. Expression of Jun and Fos proteins in ovarian tumors of different malignant potential and in ovarian cancer cell lines. Oncology reports 2009; 22(1): 177-83

26. Henke JI, Goergen D, Zheng J, et al. microRNA-122 stimulates translation of hepatitis C virus RNA. EMBO 2008; 27: 3300-3310

27. Hutvagner G, Simard MJ. Argonaute proteins: key players in RNA silencing. Nature rev 2007; vol 9: 22-32

28. Iwasaki S, Tomari Y. Argonaute-mediated translational repression (and activation). J fly 2009; 3(3): 206-209

29. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 MicroRNA family. Cell 2005; vol. 120: 635-647

30. Kefas B, Comeau L, Floyd DH, et al. The neuronal microRNA miR-326 acts in a feedback loop with Notch and has therapeutic potential against brain tumors. The J of neuroscience 2009; 29(48): 15161-15168

31. Laine J, Kunstle G, Obata T, et al. The protooncogene TCL1 is an Akt kinase coactivator. Molecular Cell 2000; 6: 395-407

32. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 3: 75(5): 843-854

33. Lee Y, Lee Y, Kim M, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO 2004; 23: 4051-4060

34. Liu X, Luo GZ, Bai X, et al. Bioinformatic analysis of microRNA biogenesis and function related proteins in eleven animal genomes. J genet genomics 2009; 39: 591- 601

35. Maniataki E, Mourelatos. A human, ATP-independent, RISC assembly machine fueled by pre-miRNA. Genes and development 19: 2979-2990

36. McNeil CM, Sergio CM, Anderson LR, et al. c-Myc overexpression and endocrine resistance in breast cancer. J steroid biochem mol boil 2006; 102: 147-155

37. Merkerova M, Belickova M, Bruchova. Differential expression of microRNAs in hematopoietic cell lineages. European J of haematology 2008; 81: 304-310

38. Monteys AM, Spengler RM, Davidson BL, et al. Structure and activity of putative intronic miRNA promoters. RNA 2010; 16: 495-505

39. Niepmann M. Activation of hepatitis C virus translation by liver-specific microRNA. Cell cycle; 8 (10): 1473- 1477

40. O´Connell RM, Chaudhuri AA, Baltimore D, et al. Inositol phosphatase SHIP1 is a primary target of miR-155. PNAS 2009; 106(17): 7113-7118

41. Okada C, Yamashita E, Lee SJ, et al. A high-resolution structure of the pre-microRNA nuclear export machinery. Science 2009; vol 326: 1275-1279

42. Okamura K, Ishizuka A, Siomi H, et al. Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. Genes and development 2004; 18: 1655-1666

43. Pallasch CP, Patz M, Park YJ, et al. miRNA deregulation by epigenetic silencing disrupts suppression of the oncogene PLAG1 in chronic lymphocytic leukemia. Blood 2009; 114 (15): 3255- 3264

44. Pedersen IM, Otero D, Kao E, et al. Onco-miR-155 targets SHIP1 to promote TNFa-dependent growth of B cell lymphomas. EMBO Mol med 2009; 1(5): 288-295

45. Pekarsky Y, Santanam U, Cimmino A, et al. Tcl1 expression in CLL is regulated by miR-29 and miR-181. Cancer Research 2006; 66: 11590-11593

46. Sachdeva M, Zhu S, Wu F, et al. P53 represses c-Myc through induction of the tumor suppressor miR-145. PNAS 2008; 106(9): 3207-3212

47. Saini HK, Griffiths-Jones S, Enright AJ. Genomic analysis of human microRNA transcripts. PNAS 2007; 104(45): 17719–17724

48. Srinivasan L, Sasaki Y, Calado P, et al. PI3 kinase signals BCR-dependent mature B cell survival. Cell 2009; 139(3): 573-589

49. Tili E, Michaille J, Cimino A, et al. Modulation of miR-155 and miR-125b levels following lipopolysacharide/TNF-alfa stimulation and thein possible roles in regulativ the response to endotoxin shock. J of imunology 2007; 179: 5082-5089

50. Trojani A, Montillo M, Nichelatti M, et al. ZAP-70, IgVh, and cytogenetics for assessing prognosis in chronic lymphocytic leukemia. Cancer biomark 2010; 6(1): 1-9

51. Wang J, Lu M, Qiu C, et al. TransmiR: a transcription factor-microRNA regulation database. Nucleic acids research 2010; 38: D119-D122

52. Whiteman HJ, Farrell PJ. RUNX expression and function in B cells. Critical review Eukaryot genes expression 2006; 16(1): 31- 44

53. Yang SH, Jaffray E, Hay TR, et al. Dynamic interplay of the SUMO and ERK pathways in regulating Elk-1 transcriptional activity. Molecular cell 2003; vol.12: 63-74

54. Yi R, Qin Y, Macara IG, et al. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes dev 2003; 17: 3011-3016

55. Yin Q, Wang X, McBride J, et al. B-cell receptor activation induces BIC/miR-155 expression through a conserved AP-1 element. J of biological chemistry 2008; 283(5): 2654-2662

56. Yu J, Wang F, Yang GH, et al. Human microRNA clusters: Genomic organization and expression profile in leukemia cell lines. Biochem and biophys res communications 2006; 349: 59-68

57. Ma JB, Ye K, Patel DJ. Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. Nature 2004; 429(6989): 318-322