The impact of Mir-9 regulation in normal and malignant hematopoiesis

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Abstract

MicroRNA-9 (MiR-9) dysregulation has been observed in various cancers. Recently, MiR-9 is considered to have a part in hematopoiesis and hematologic malignancies. However, its importance in blood neoplasms is not yet well defined. Thus, this study was conducted in order to assess the significance of MiR-9 role in the development of hematologic neoplasia, prognosis, and treatment approaches. We have shown that a large number of MiR-9 targets (such as FOXOs, SIRT1, CCND1, ID2, CCNG1, Ets, and NFkB) play essential roles in leukemogenesis and that it is overexpressed in different leukemias. Our findings indicated MiR-9 downregulation in a majority of leukemias. However, its overexpression was reported in patients with dysregulated MiR-9 controlling factors (such as MLLr). Additionally, prognostic value of MiR-9 has been reported in some types of leukemia. This study generally emphasizes on the critical role of MiR-9 in hematologic malignancies as a prognostic factor and a therapeutic target.

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Introduction

Leukemia is caused by the uncontrolled proliferation of hematopoietic cell lineages. Different factors such as genetic backgrounds, viruses, chemicals, and radio waves induce leukemia. Leukemia is the sixth leading cause of cancer related mortality.[1] Radiotherapy, chemotherapy, stem cell transplantation, and immunotherapy are the most common therapeutic approaches in leukemia treatment.[2] However, the lack of an effective and relatively non-toxic treatment is obvious despite the improvement in the knowledge in respect to leukemia pathogenesis. In addition to different gene mutations, various epigenetic factors such as non-coding RNAs also play an essential role in the development of leukemia. Micro-RNAs (miRs) are the most well-known non-coding RNAs. miRs are small single-strandedendogenous RNAs with important roles in gene regulation through disrupting the expression of target genes.[3] Additionally, these 19-22 bp nucleotide molecules are involved in different biological processes such as growth, proliferation, differentiation, and cell death.[4,5] Different experimental studies proved the critical role of miRs in cancer development, progression, and metastasis.[6,7] Also, miRs play critical roles in different processes of hematopoiesis such as lineage differentiation and commitment, apoptosis, and cell function. Therefore, any change in miRs expression signature can have make a significant impact on different biological processes of cells.[8,9]

MiR-9 was discovered as a vital regulator of organ growth and neurogenesis.[10] Subsequently, further studies showed that MiR-9 dysregulation might occur in various cancers. Therefore, MiR-9 is suggested to be an oncogene or a tumor suppressor. Three independent MiR-9 genes are identified in humans, including MiR-9-1 on chromosome 1, MiR-9-2 on chromosome 5, and MiR-9-3 on chromosome 15.[11] Overexpression of MiR-9 has consequently been observed in Hodgkin's lymphoma, primary brain tumor, CDX2-negative gastric cancer, and endometrial cancer, while its downregulation has been observed in cervical, colorectal, lung, ovarian, and hepatocellular cancers, which suggests its oncogenic roles.[12-21] On the other hand, MiR-9 silencing through the methylation of cytosine-phosphate-guanine Islands (CPG) was observed in many cancers, which clarifies its tumor suppression features.[13,21-25] Furthermore, downregulation of MiR-9 is a marker of poor prognosis in cervical and lung cancers as well as acute lymphoblastic leukemia (ALL).[18,21,25] Therefore, it seems that MiR-9 has a two-faced role in cancers as tumor suppressor and tumor inducer. MiR-9 has been detected in hematopoietic stem cells (HSCs) and progenitors. Moreover, its expression has been shown to increase during hematopoietic differentiation. However, the

aberrant expression of MiR-9 clearly steps up terminal myelopoiesis and induces apoptosis. Conversely, its inhibition blocks myelopoiesis.[26] Finally, these observations raise the question of which of the MiR-9 faces are enrolled in leukemia. Therefore, this study aims to evaluate the distinct role of MiR-9 in leukemia pathogenesis. We have attempted to show the prognostic impact of MiR-9 in leukemia. The potential of MiR-9 as a therapeutic target in leukemia was also reviewed.

MiR-9 and cell cycle

Leukemia is derived from the accumulation of mutations in oncogenes or tumor suppressor genes as well as disruption of balance between proliferation and differentiation in hematopoietic progenitor cells' pool.[27,28] Hematopoietic homeostasis causes balanced proliferation and differentiation in different levels of hematopoiesis such as HSCs self-renewal, progenitors' proliferation, and terminal differentiation of mature blood cells. In fact, leukemic transformation is a type of disrupted balance in which proliferation predominates differentiation.[29] Thus, the cell cycle regulators play a key role in the control of these processes.[30] Cell cycle is controlled by various transcription factors; therefore, the heterogeneity of stem cells depends on the type of transcription factors expressed in stem cells.[31] Cell cycle is a highly precise process and occurs in response to specific cell or tissue requirements. Cyclin-dependent kinases (Cdks) and their cyclins, which are considered as the second part of Cdk holoenzymes, are among the most important regulators of cell cycle. Cdks include nine proteins, which are activated or inactivated through their threonine phosphorylation by Cdk activator kinase or dephosphorylation by cdk phosphatase, respectively. Based on their function, cyclins are divided into three categories of G1-S cyclins, S cyclins, and M cyclins.[32] Cdks activity is regulated at transcriptional and post-transcriptional levels.[33] It has been indicated that miRs play an important role in cell cycle regulation, and several miRs have been reported to regulate cell cycle.[34] MiR-9 mostly targets proteins that are involved in cell cycle. Therefore, it has been hypothesized that one of the most important tumorigenic mechanisms of MiR-9 dysregulations may be done through cell cycle disruption. Subsequently, the role of miR-9 targets that act as cell cycle regulators will be discussed in normal and malignant hematopoiesis.

Cell cycle induction

Cyclin D1 activates its cognates (*i.e.* Cdk4/Cdk6) in response to mitogenic growth factors and leads to cell cycle progress from the beginning to mid G1 phase. Cyclin-D1 dependent kinase inactivates retinoblastoma (Rb) tumor suppressor through a phosphorylation-dependent mechanism.[35] Cyclin-D1, which is a target of miR-9, plays an essential role in normal hematopoiesis in addition to its important role in cell cycle.[36,37] Although cyclin-D1 expression in Acute Myeloid leukemia (AML) patients is not significantly different with normal individuals, patients with acute lymphoblastic leukemia (ALL) show a remarkable overexpression of Cyclin-D1.[38] Moreover, its overexpression has been reported in the accelerated phase of CML.[39]

Anaphase-promoting complex/cyclosome (APC/C) is a multifunctional ubiquitin-protein ligase regulating the cell cycle. APC/C activation depends on cell division cycle protein 20 (Cdc20) and Cadherin-1 (Cdh1). Cdc20 activates APC/C in early stages of mitosis while Cdh1 (also known as E-cadherin) has a significant role in late stages of mitosis and G1/S transition.[40] APC/Cdh1 plays a role in genomic stability and cell cycle transition, as well as regulating cell differentiation in addition to its role in cell cycle control. Cdh1 downregulation has been indicated in several hematologic neoplasias and solid tumor cell lines.[41] Cdh1 expression is essential in erythroblastic maturation as its expression decreases in leukemic blast cells, and Cdh1 expression reduction has been detected in various acute and chronic leukemias such as AML, ALL , chronic myeloid leukemia (CML), and chronic lymphocytic leukemia (CLL).[42,43] It has been reported that Cdh1 downregulation is caused by hypermethylation in 70% of cases.[42] Cdh1 is reported to be a direct target of MiR-9.[24] Meanwhile, MiR-9 can be another important regulator of Cdh1.

ETS proto-oncogene 1 (Ets-1) is another target of MiR-9[36] as well as a prototype of ETS transcription factors family that is involved in many biological functions.[44] Ets-1 facilitates G1/S-phase transition through the upregulation of Cyclin E and CDK2 genes.[45] Additionally, Ets-1 inhibits $CD34^+$ cell proliferation by reducing cylin D3 expression.[46] Therefore, Ets-1 plays an important role in cell cycle regulation. On the other hand, Ets-1 has been shown to be capable of inducing apoptosis in tumor cells through regulating caspase-1 expression.[47,48] According to the dual role ofEts-1 in various cells, the accurate regulation of its expression is of importance in cell fate. Assessment of the Ets-1 role in hematopoiesis indicates its importance in proliferation and differentiation of different blood cells. Ets-1 downregulation leads to granulocytes differentiation,[49] and it has been reported to be a differentiation regulator of plasma cells as well as B cells. Increase in Est-1 expression is known to be required for B-cell maturation; however, its expression should be reduced for plasma cell differentiation.[50] Also, the role of this transcription factor has been evaluated in regulating erythroid/megakaryocyte differentiation. Est-1 overexpression in hematopoietic progenitors blocks erythroid differentiation and induces the differentiation progress towards megakaryocyte lineage.[51] The important point about Ets-1 is its overexpression in AML patients' blasts and erythroleukemia cell lines.[49,51] Additionally, it has been identified that the overexpression of MiR-9 leads to reduced expression of Ets related gene (ERG), a poor prognosis marker in AML, which consequently increases remission and reduces the diseases relapse.[52]

Nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) transcription factor family that includes NFkB1, NFkB2, Rel-a, Rel-b, and Rel-c stimulates the expression of proteins involved in cell growth, proliferation, differentiation, as well as immune and inflammation response.[53] Increase in NFkB activity leads to cell cycle progression through transcriptional activation of cyclin D1 gene and increasing Cdk-D1 activity. Moreover, this transcription factor inhibits terminal differentiation in mammalian cells.[54] NFkB1 plays a key role in hematopoiesis; consequently, this transcription factor contributes to the differentiation and activation of macrophages, granulocytes, osteoblasts, dendritic cells, and erythrocytes, and the involvement of NFkB1 has been demonstrated in lymphopoiesis, too.[55] It seems that NFkB also plays a role in apoptosis inhibition. Recent studies illustrated that NFkB inhibition could promote apoptosis in granulocytes and lymphocytes.[55,56] Although NFkB expression is considered to be an important factor in survival of normal cells, its apoptosis inhibitor role in tumor cells can be an obstacle in the treatment of leukemia patients. It has been indicated that NFkB inhibition can increase apoptosis in leukemic cell lines and ALL patients.[57,58] Also, the overexpression of NFkB has been shown in hematologic malignancies, particularly AML.[59] Several drugs have been suggested for NFkB inhibition and apoptosis induction in cancer cells.[57-59] Because of the regulatory effect of MiR-9 on NFkB, its induction might affect NFkB expression.[60] However, further experiments are required to prove this hypothesis.

Serum response factor (SRF) is a transcription factor that regulates the expressions of various genes and regulates different cellular activities such as proliferation, differentiation, angiogenesis, migration, and apoptosis.[61] It has been reported that the constitutive expression of SRF protein is sufficient to initiate cell cycle. Moreover, it was shown that PI3K-dependent cell cycle progression was associated with SRF activation and subsequent SFR-related gene expression.[62] Additionally, SRF has been recognized as an essential factor in HSCs homeostasis. Furthermore, it seems that SRF plays a role in AML through its co-activator, *i.e*. T-cell differentiation protein (MAL).[63] This transcription factor is also one of the MiR-9 targets.[64] According to our knowledge, there are few studies with respect to SRF evaluation in hematologic neoplasms. Therefore, SRF expression in these patients can give us valuable information about its role in blood malignancies.

Cell cycle arrest

Forkhead box protein O1 **(**FOXO1), a member of *Forkhead* family transcription factors, regulates the expressions of a large number of genes that play a critical role in cell cycle and apoptosis.[65] FOXO induces $p21^{Cip1}$ expression, decreases cyclin D1 and D2 expressions and also increases cyclin G2 and P130 expression. Cell passage from G0 to G1 phase requires increase in cyclin D expression and $p21^{Cip1}$ degradation. However, the increase in cyclin G2 and P130 expression is seen in quiescence cells.[66] Thus, FOXO's function is to keep the cell in G0 phase, which leads to cell cycle arrest. FOXO induces arrest in G2 through regulating the expression of Growth Arrest and DNA Damage-inducible 45 (GADD45).[67] and is also essential in the maintenance of hematopoietic cells. In addition to FOXO1, FOXO3, which is another member of this family, regulates a cell cycle inhibitor factor called $p27^{Kip1}$ and also downregulates the expression of CDK2, cyclin D1, and proliferating cell nuclear antigen (PCNA).[68] However, Akt signaling pathway is considered as the most important regulator of these factors. Recently, other pathways have been noticed in hematopoiesis.[69] FOXOs areoverexpressed in 40% of AML patients regardless of their genetic subtypes, and their expression is required to maintain leukemic initiating cells (LICs). It has been shown that FOXO inhibition can lead to myeloid maturation and subsequent AML cell death.[70] Moreover, FOXO1 overexpression is reported to be a key factor in BCR-ABL1-independent drug resistance in CML patients.[71] Recently, studies have shown that B-ALL cells have a high expression level of FOXO1 which regulates their survival.[72] Hence, FOXO1 is proposed to be a therapeutic target in these neoplasias. Nevertheless, FOXO3 plays various roles in different hematopoietic neoplasms but its expression increases in AML, and it is suggested to act as an oncoprotein in AML patients. BCR-ABL1 positive patients showed a downregulation of FOXO3.[73,74] FOXO1 and FOXO3 are targets of MiR-9,[75] and these findings generally raise the question of whether inducing MiR-9 expression through reducing FOXO expression affects apoptosis process in leukemic cells. The answer to this question requires experimental studies.

Cyclin G1 (CCNG1), a P53 target gene, operates in P53-dependent and independent manners.[76] CCNG1 is associated with CDK5 and non-CDK-serine/threonine kinase (cyclin G associated kinase). It acts as an oncogene, and its overexpression has been observed in human cancer cells. Also, this protein is involved in G2/M arrest induced by DNA damage.[77,78] However, the distinct role of CCNG1 in hematopoiesis and hematologic malignancies has not been defined, and the authors reported that its overexpression in acute leukemia patients was associated with apoor prognosis.[79] CCNG1 has been known as a validated target of MiR-9.[80]

Transforming Growth Factor β1 (TGF-β1) is a member of a growth factors family that inhibits cell cycle in various types of human cells. TGF-β1 arrests cell cycle at G1 through *smads*, which regulates different transcriptional targets including C-myc. C-myc downregulation induces $p15^{INK4b}$, which is a Cdk4-cyclin D inhibitor. Furthermore, TGF-β1 inhibits cdk2-cyclin through $p27^{Kip1}$.[81] Therefore, TGF-β1 arrests the cells in G1 phase through the aforementioned mechanisms. This growth factor inhibits the proliferation of quiescent hematopoietic cells and stimulates the differentiation of late progenitors to erythroid and myeloid cells. Additionally, TGF-β1 leads to tumor growth and survival through affecting the tumor microenvironment.[82] Aberrantlyincreased production of TGF-β1 promotes leukemia development through inducing fibrosis in bone marrow (BM). In these circumstances, additional secretion of TGF-β1 from leukemic cells, monocytes, and megakaryocytes stimulates collagen synthesis in fibroblasts deposited in BM.[83] The overexpression of MiR-9-5p inhibits TGF-β1-mediated differentiation of fibroblasts, so that MiR-9 induction drastically decreases fibrogenesis. But, interestingly, TGF β1 induces MiR-9 expression.[84] It has been thought that there is a feedback loop between TGF β1 and MiR-9, which means that it can either be a target of MiR-9 or induce MiR-9 expression.

Other factors

REST Corepressor 1(RCOR1), another target of MiR-9, is one of the most important transcription co-repressors expressed in HSCs as well as progenitors, which is also involved in progeny differentiation. The role of RCOR1 has been recently studied in normal hematopoiesis. It has been reported as an essential factor in normal differentiation of myeloerythroid progenitors, which regulates self-renewal activity in monocytes.[85,86] More studies should be done on RCOR1 expression in leukemia patients in order to determine its role in malignant hematopoiesis.

MiR-9 dysregulation in leukemia

Few studies have been done with respect to MiR-9 expression in blood neoplasias. The findings are apparently inconsistent, and it is not easy to draw final conclusions. Chen *et al*. proved that MiR-9 is an essential oncogene and can even be regarded as a therapeutic target in patients with mixed lineage leukemia-rearranged (MLLr) AML.[87] Conversely, Emmrich *et al*. argued that MiR-9 is a tumor suppressor in AML patients with t(8; 21).[88] Weinder *et al*. reported that MiR-9 induction by drugs in AML blasts may improve the disease outcomes.[52] Theprognostic role of MiR-9 in patients with ALL is conflicting. Otero *et al*. emphasized that MiR-9 is hypermethylated in 54% of ALL patients and its consequent reduction is associated with a poor prognosis.[25] Sugita *et al*. presented documents that MiR-9 overexpression was observed in 20% of patients with ALL and that it was a poor prognosis predictor in these patients.[89] In order to clarify these paradoxical studies, a review was carried out and showed that AML patients with favorable cytogenetic findings such as t(8; 21), inv(16), and t(15; 17) had a low expression level of MiR-9 while AML patients with adverse or intermediate cytogenetic risk showed MiR-9 overexpression.[90] Therefore, genetic aberrations could determine the prognostic role of MiR-9 in ALL patients. Additionally, determining the prognostic and pathogenic roles of validated targets of MiR-9 can designate its role in the development, progression, and outcome of leukemia.

The overexpression of a majority of MiR-9 targets strongly correlates with the development of hematologic malignancies. These targets include Peripheral myelin protein 22 (PMP22),

Sirtuin 1 (Sirt1), ETS Proto-Oncogene 1 (Ets1), Stathmin 1 (STMN1), and Caudal Type Homeobox 2 (CDX2).[20,36,49,91-97] PMP22 is an oncogene, which is observed in neoplastic processes of prostate and breast cancers. This oncogene plays an important role in leukemic stem cells growth and survival.[98-100] Liu *et al.* showed that PMP22 expression level in cells isolated from CML patients was significantly higher than the control group. They also proved that PMP22 knockdown could inhibit the proliferation of CML cells, decrease bcl-xl expression, increase caspase-3 expression, and finally increase neoplastic cells' apoptosis.[97]

SIRT1 is a deacetylase that selectively deacetylates histone H4K16 and H1K26, which subsequently plays a role in gene silencing and heterochromatin formation.[101-103] SIRT1 affects various cell processes through affecting different genes such as p53, FOXO1, FOXO3a, NF-kB, C-MYC, N-MYC, and E2F1 expressions.[104,105] SIRT1 expression increases in various blood malignancies such as ALL, CLL, CML, and AML.[106-109] Moreover, recent experiments indicated that SIRT1 inhibition by a drug or through RNA interference leads to disease remission via increased expression of p53.[106-109]

Ets-1 is a member of ETS family of transcription factors. Ets-1 plays an important role in cell proliferation, apoptosis, transformation, differentiation, angiogenesis, and hematopoiesis. Ets-1 expression increases in various human tumors and has prognosis value in malignancies.[110] Increase in Ets-1 expression has been observed in malignant T-cells as well as cells isolated from AML patients.[111-113]

STMN1 is a microtubules destabilizer that has an important role in cell cycle progression, chromosome segregation, clonogenicity, cell movement, and survival. Studies suggest that STMN1 is overexpressed in malignant hematopoietic cells and that its inhibition reduces the proliferation of leukemic cell line.[96]

CDX2 gene is located on chromosome 13q12.12 and encodes a transcription factor, which plays a role in hematopoiesis and embryonic organogenesis in vertebrates. Initial studies showed that theaberrant expression of CDX2 is probably associated with metaplasia in gastrointestinal tract tumors.[114,115] Consequently, research on hematologic malignancies provided interesting results, and numerous studies showed that CDX2 gene was aberrantly expressed in a variety of hematologic malignancies such as ALL, AML, and CML.[116-118]

Basic-helix-loop-helix (bHLH) family of transcription factors has a role in cell differentiation, including the differentiation of B and T lymphocytes. Inhibitor of DNA binding (ID) is another group of proteins belonging to HLH family and classified as ID-1 to ID-4 types. ID proteins attach to both classes of b-HLH and inhibit their binding to DNA.[119] Among ID proteins, ID-2 is known as the MiR-9 target.[120] ID-2 (but not ID-3) can attach to all the three pRb family members including pRb, p10, and p130 to abolish their growth-suppressing activity. Therefore, ID2 acts as an antagonist of several tumor suppressors[121] and has an important role in hematopoiesis. It has been shown that ID2 is expressed in mature myeloid blast cells and that its expression is increased in terminal differentiation of myeloid cells.[122] ID2 expression significantly increases in acute leukemias.[123] Recently, some pieces of evidence have suggested the pro-survival function of ID2 in chronic lymphocytic leukemia cells.[124]

MiR-9 regulation

There are few studies about MiR-9 regulatory factors. Davila et al. recently demonstrated that MiR-9 promoter contains two binding sites for myocyte enhancer factor 2C (Mef2C) transcription factor and that its exclusive inhibition reduces the activity of MiR-9 promoter.[125] Mef2C oncogenic role has been reported in hematological cancers, and it is identified as a well-characterized oncogene.[126] Aberrant Mef2C expression is observed in 20% of myeloid malignancies, and its expression increase is associated with a poor prognosis in AML.[127,128] Increase in Mef2C expression is one of the prominent features of immature T-cell acute lymphoblastic leukemia.[129] The Mef2C is activated by ISL LIM Homeobox 1 (ISL1), and Growth Factor Independent 1B Transcriptional Repressor (GFI1B), Histone deacetylases (HDACs), homeodomain interacting protein kinase 2 (HIPK2), NK2 Homeobox 5 (NKX2-5) are major inhibitors of Mef2C.[130] Mef2C activation is regulated by p38 MAPK-dependent phospho-

Table 1. Some known drugs that interact with MiR-9 regulators.

Gene	Drug	Interaction type	Drug class	FDA approval
HDACs	BELINOSTAT	Inhibitor	Antineoplastic	Peripheral T-cell lymphoma
	ROMIDEPSIN	Inhibitor	Antineoplastic	Cutaneous T-cell lymphoma, Peripheral T-cell lymphoma
	CUDC-101	Inhibitor	Antineoplastic	Not approved
	PANOBINOSTAT	Inhibitor	Antineoplastic	Multiple myeloma
	CHEMBL1213492	Inhibitor	Antineoplastic, Anti-inflammatory agent	Not approved
	CHEMBL1851943	Inhibitor	Antineoplastic	Not approved
	VORINOSTAT	Inhibitor	Antineoplastic	Cutaneous T-cell lymphoma
	ENTINOSTAT	Inhibitor	Antineoplastic	Not approved
	VALPROIC ACID	Inhibitor	Anticonvulsants	Approved
	ABEXINOSTAT	Inhibitor	Antineoplastic	Not approved
CREB	ADENOSINE PHOSPHATE (Vitamin B8)	Activator	Nutritional supplementa-tion	N/A
ELK1	GANCICLOVIR	N/A	Anti-Viral	Approved
RUNX1	CYTARABINE	N/A	Antineoplastic	Approved
	DOXORUBICIN	N/A	Antineoplastic	Approved
	DIPHENHY-DRAMINE HY-DROCHLORIDE	N/A	Antineoplastic	Approved
	Methacholine	N/A	Bronchial air-way hyperreac-tivity	Approved
SMAR-CA4	TRETINOIN	N/A	Antineoplastic	Approved
	VINORELBINE BASE	N/A	Antineoplastic	Approved

HDACs, Histone deacetylases; CREB, cAMP response element binding; ELK1, ETS transcription factor; RUNX1, runt-related transcription factor 1; SMARCA4, SWI/SNF related, matrix associat-ed, actin dependent regulator of chromatin, subfamily a, member 4.

rylation as well as calcium-dependent calcineurin-calmodulin pathways.[131] P38 MAPK activates Mef2C through phosphorylating Mef2 transcription activation domain, while calcium calmodulin-dependent protein kinase (CaMK) stimulates Mef2 activity through dissociating class II histone deacetylases (HDACs) from DNA binding-domain. The maximum activity of Mef2 is only achieved by HDACs inhibition through CaMK signaling pathway.[132]

The role of P38 MAPK activity in hematologic malignancies is not well understood.[133] According to our knowledge, P38 MAPK activity has only been shown in B-CLL cell survival.[134] However, the increase in expression and enzymatic activity of HDACs has been reported in various hematologic malignancies. Bradbury *et al*. showed that the increase in HDAC2 expression can be observed in most AML patients.[135] Moreover, its overexpression in T-ALL and CLL has also been reported.[136] Additionally, the role of HDACs inhibitors (HDACi), especially HDAC2i, in blood neoplasia treatment has been shown.[137-139] Therefore, it seems that MiR-9 dysregulation occurs as a result of aberrantly increased HDACs expression or activity that subsequently decreases Mef2C activity. Moreover, it is thought that one of the important mechanisms of hematologic malignancies treatment by HDAC2 inhibitors can be achieved by restoring Mef2c activity, which consequently increases MiR-9 expression. Nevertheless, experimental studies are required to confirm this hypothesis.

Cyclic adenosine monophosphate response-element binding protein (CREB), a leucine zipper transcription factor, regulates various cellular processes such as proliferation, differentiation, and cell survival.[140] It has been reported that CREB can bind to MiR-9 promoter and induce its expression. Moreover, it has also been shown that CREB knockdown leads to approximately 60% decrease in MiR-9-2 level.[141] The role of CREB in normal and malignant hematopoiesis has been evaluated by many studies.[142-145] Overexpression of CREB leads to increased proliferation and survival of myeloid cells. Nevertheless, CREB induction also promotes myeloproliferative disease. Therefore, CREB can be regarded as a proto-oncogene regulator of hematopoiesis that contributes to the leukemia progression.[143] BM samples of AML or ALL patients showed a higher expression level of CREB than control BM, nonleukemic patients or during leukemia remission.[142] Moreover, it was also shown that CREB overexpression could predict early relapse and a poor outcome in AML patients.[143-145] CREB expression alteration leads to MiR-9 dysregulation and could be considered as an important part of CREB leukemia promoting process. Other regulators are reported to control MiR-9 expression. Snyuk *et al*. reported that Ectopic viral integration site 1 (EVI1) inhibits MiR-9 through MiR-9 promoter hypermethylation.[87] The transcription activators of EVI1 are ELK1, RUNX1, and SMARCA4, which can be targeted by some approved and non-approved drugs (Table 1). Furthermore, MLL fusion protein directly targets MiR-9 and significantly increases its expression.[87]

Table 2. Some of the Mir-9 targets dysregulated in leukemia.

Gene	Location	Function	Expression in leuke-mia	Ref.
CDH1	16q22.1	It is a calcium-dependent cell-cell adhesion protein	AML↓ ALL↓ CML↓	58,151
ETS1	11q24.3	Functions either as a transcriptional activator or repressor of numerous genes	AML ↑	49
NFKB1	4q24	Activated NFKB translocates into the nucleus and stimulates the expression of genes in-volved in a wide variety of biological func-tions.	AML ↑ ALL ↑ CML ↑ CLL ↑	59
FOXO1	13q14.11	It may play a role in myogenic growth and differentiation	AML ↑ ALL ↑ CML ↑	70-72
CDX2	13q12.2	Major regulator of intestine-specific genes involved in cell growth and differentiation. Aberrant expression of this gene is associated with intestinal inflammation and tumorigene-sis	AML ↑ ALL↑ CML↑	116-118
CCNG1 (cyclin G1)	5q34	It is a member of the cyclin family and con-tains the cyclin box that may be regulated by P53	Acute leukemia ↑	79
SIRT1	10q21.3	May function as an intracellular regulatory protein with mono-ADP-ribosyltransferase activity	Leukemia ↑	96
ID2	2p25.1	Inhibitor of DNA binding family, members of which are transcriptional regulators that contain a helix-loop-helix (HLH) domain but not a basic domain	Myeloid and Lymphoid leukemia ↑	123
FOXO3	6q21	Functions as a trigger for apoptosis through expression of genes necessary for cell death	ALL ↑ CML ↑ AML ↑	70-72
CCND1	11q13.3	Functions as a regulatory subunit of CDK4 or CDK6, the activity of which is required for cell cycle G1/S transition	ALL ↑ AML CML↑	38-39

ETS1, ETS proto-oncogene 1; NFKB1, nuclear factor kappa B subunit 1; FOXO1, orkhead box O1; CDX2, caudal type homeobox 2; CCNG1, cyclin G1; SIRT1 sirtuin 1; ID2, inhibitor of DNA binding 2; FOXO3, forkhead box O3; CCDN1, cyclin D1.

In silico analysis of MiR-9 in leukemia

In the next part, we have mined MiR-9 expression data from public databases (Figure 1). Recently, Tan *et al*. performed a non-coding RNA profiling array in order to show MiR signature in leukemic cells compared to normal cells.[146] We used the available data on GEO database by mining the expression of MiR-9. The results showed that myeloid leukemia cells had a significantly lower expression level of MiR-9 compared to normal myeloid cells (P=0.045) (Figure 2). In another analysis, we have compared the leukemic cells of B-ALL patients with normal B-cells. Similarly the MiR-9 expression was lower in ALL leukemic cells but the differences was not statistically significant (P=0.55) (Figure 3). Moreover, it was found that MiR-9 expression was significantly lower in patients with T-ALL compared with normal T-cells (t=–2.068, P=0.048) (Figure 4). Therefore, it seems that MiR-9 could be considered as a tumor suppressor in leukemic patients.

After determining the expression of MiR-9 in different leukemias, we have tried to find the relationship of MirR-9 expression with survival of leukemia patients using PROGmiR database.[147] As shown in Figure 5, a high expression level of MiR-9 significantly reduced survival rate of AML patients [P=0.049, hazard ratio=1.07 (1-1.14)]. This finding might show the prognostic value of MiR-9 expression in patients with AML. More studies are needed to find out the prognostic value of MiR-9 in other leukemia types.

Discussion and future prospective

miRs regulate gene expression, and any alteration of them may lead to cancer development.[148] Previous studies prove important roles of miRs in cancer progression.[149,150] For a long time, MiR-9 was considered as an important regulator in neurogenesis and nerve tissue development.[10] This was followed by further studies, which investigated MiR-9 dysregulation role in various cancers. The distinct role of MiR-9 is related to cancer type, so MiR-9 plays a tumor suppressor role in some cancers and an oncogenic role in others.[11] Recently, MiR-9 expression is discussed in various hematologic neoplasms with paradoxical roles. To solve this problem, we initially studied validated MiR-9 targets in cell cycles of normal and malignant hematopoiesis. The results indicated that MiR-9 targeted cell cycle-promoting genes such as cyclin D1, Ets, NFkB, Cdh1, SRF, as well as cell cycle inhibitors such as FOXO1

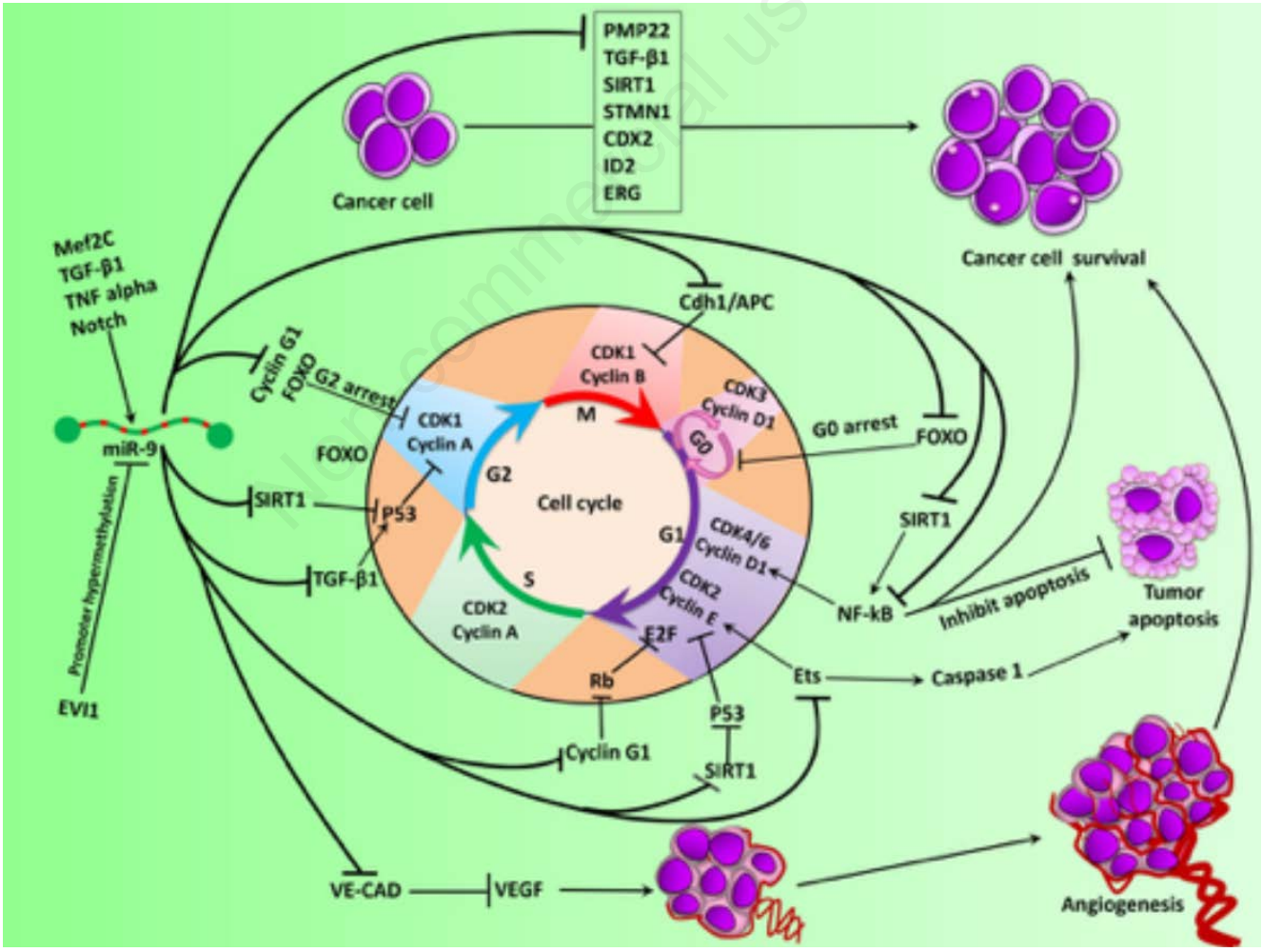

Figure 1. Summary of miR-9 targets and functions. MiR-9 affects different cell cycle mole-cules, cancer cell survival, apoptosis, and angiogenesis. Hence, maybe miR-9 targeting mole-cules can explain tumor suppressor or tumor initiator effect of miR-9.

and Cyclin G1.[24,36,37,60,64,75,80] Overall, MiR-9 has a dual role in cell cycle regulation. These findings point out that MiR-9 dysregulation disturbs normal cell cycle. MiR-9 targets dysregulation, which has been proven in myeloid and lymphoid neoplasms. Therefore, MiR-9 dysregulation may lead to leukemia development through disrupting the expression of its targets. Interestingly, among the validated MiR-9 target genes dysregulated in hematologic malignancies, only Cdh1 is downregulated in three leukemic lineages (Table 2).[151,152] However, it has been reported that Cdh1 hypermethylation is the main reason for its downregulation and that the role of other epigenetic regulators is inconsiderable.[42] Based on these findings, MiR-9 downregulation and subsequent overexpression of its targets is more likely to cause leukemic progression and might act as a tumor suppressor in hematologic malignancies. Our analysis on mined data from public databases also confirmed these findings later. However, oncogenic role of MiR-9 in hematologic malignancies was also reported in patients with MLLr.[87] Thus, there are two hypotheses as follows: i) The tumor suppressor or oncogenic role of MiR-9 is likely associated with the involved cell lineage and other genetic abnormalities such as MLLr; ii) It is also likely that excessive increase or decrease (*i.e.*, dysregulation) of MiR-9 leads to leukemia development through disrupting normal hematopoiesis. Another important finding is that MiR-9 can be utilized as a prognostic factor in hematologic neoplasms. Moreover, MiR-9 pharmacological induction can be used as a therapeutic target in malignancies when MiR-9 targets expression changes. Experimentally, MiR-9 regulation was shown to be a good therapeutic target in AML patients.[52]

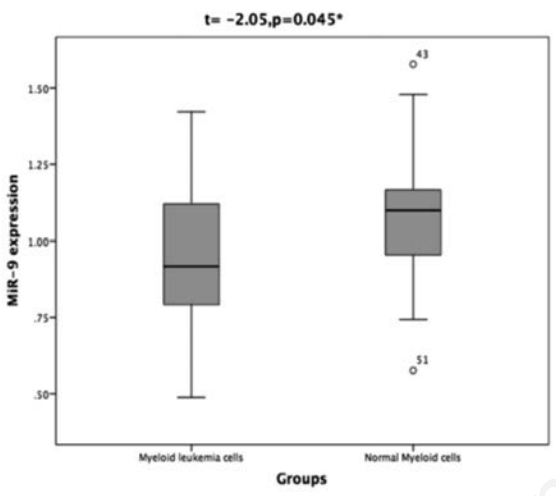

Figure 2. MiR-9 expression comparison in myeloid leukemia cells and normal myeloid cells.

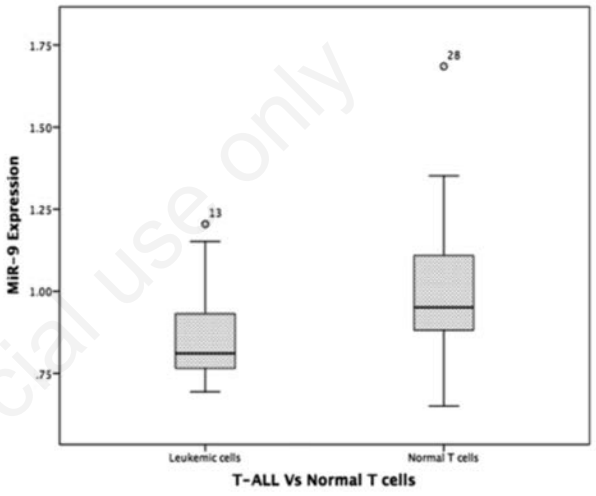

Figure 4. Comparison of MiR-9 expression in T-ALL leukemic cells and normal T-cells.

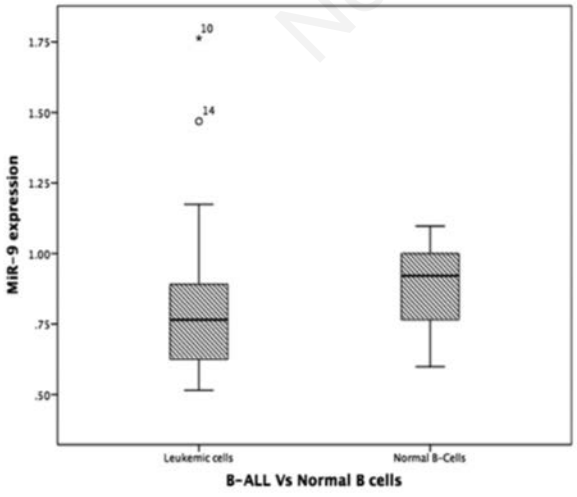

Figure 3. MiR-9 expression in B-ALL leukemic cells compared to normal B-cells.

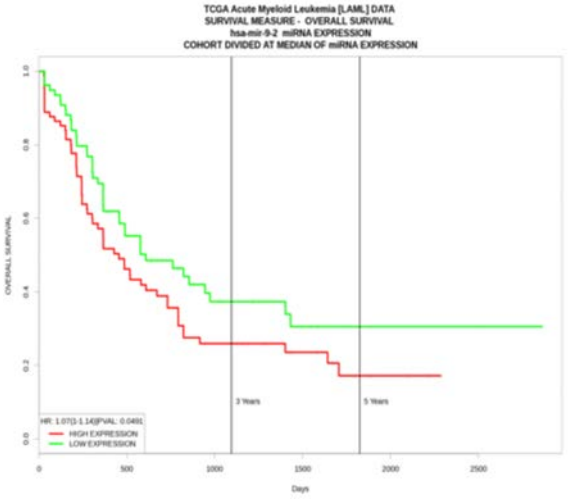

Figure 5. MiR-9 expression significantly reduces overall survival in AML patients.

Conclusions

This study generally indicates that MiR-9 dysregulation leads to leukemia development through various targets. Tumor suppressor or oncogenic role of MiR-9 is different in various leukemia subtypes. Finally, MiR-9 has a prognostic value in leukemia, and its prognostic role has been investigated in AML and ALL.[22,90] Thus, MiR-9 has an important role in hematological malignancies. In order to accurately determine the extent of MiR-9 involvement in the development of myeloid neoplasms, it is suggested to evaluate the effect of MiR-9 induction on different hematological malignancies and subsequently assess the expression of its target genes in further studies.

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