Targeting forkhead box transcription factors FOXM1 and FOXO in leukemia (Review)

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Abstract. Deregulation of forkhead box (FOX) proteins has been found in many genetic diseases and malignancies including leukemia. Leukemia is a common neoplastic disease of the blood or bone marrow characterized by the presence of immature leukocytes and is one of the leading causes of death due to cancer. Forkhead transcription factors, FOXM1 and FOXO family members (FOXOs), are important mediators in leukemia development. Aberrant expression of FOXM1 and FOXOs results in leukemogenesis. Usually the expression of FOXM1 is upregulated, whereas the expression of FOXOs is downregulated due to phosphorylation, nuclear exclusion and degradation in leukemia. On the one hand, FOXOs are bona fide tumor suppressors, on the other hand, active FOXOs maintain leukemia stem cells and stimulate drug resistance genes, contributing to leukemogenesis. FOXM1 and FOXOs have been proven to be potential targets for the development of leukemia therapeutics. They are also valuable diagnostic and prognostic markers in leukemia for clinical applications. This review summarizes the present knowledge concerning the molecular mechanisms by which FOXM1 and FOXOs modulate leukemogenesis and leukemia development, the clinical relevance of these FOX proteins in leukemia and related areas that warrant further investigation.

Contents

1. Introduction

Leukemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase in immature leukocytes called 'blasts' which are arrested in the early phases of differentiation. Most leukemias show non-random chromosomal abnormalities, of which the majority are chromosomal translocations. These genetic lesions cause activation of proto-oncogenes and inactivation of tumor-suppressor genes, ultimately resulting in leukemogenesis. The molecular pathological alterations in the disease impair the regulation of normal cellular processes, such as cell differentiation, cell proliferation, cell cycle progression and cell death. Leukemia is a common malignant disease and can occur in individuals at any age. Thousands of people died from leukemia every year around the world. There are four major types of leukemia, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). Acute leukemia is characterized by a rapid clinical progression and accumulation of malignant blood cells, whereas chronic leukemia is characterized by a slow clinical progression and an increase in relatively mature but abnormal leukocytes. Lymphoblastic and lymphocytic leukemias involve lymphocytes. Myeloid leukemia affects red blood cells, some leukocytes and platelets. Epidemiologic, genotypic and animal model data all indicate a multistep and complicated oncogenic process of leukemia. Notably, the diversifying genetic and molecular alterations that drive malignant transformation of blood cells critically contribute to the pathogenesis of leukemia and the heterogeneity of the disease. Therefore, understanding the genetic basis and molecular events of various leukemias may provide new insights into leukemia diagnoses, prognoses and therapies.

Forkhead box (FOX) proteins are a superfamily of evolutionarily conserved transcriptional factors which play

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significant roles in a wide variety of cellular processes, such as differentiation, proliferation, cell cycle progression, apoptosis, metabolism and migration. They are characterized by a forkhead or winged helix DNA-binding domain. Fifty human FOX proteins, which are further divided into 19 subfamilies (FOXA to FOXS) according to their sequence homology inside and outside the DNA-binding domain, have been identified (1). Human FOX proteins are a large family, displaying a remarkable functional diversity and regulation complexity. Besides being transcription factors, FOX proteins are also pioneer factors, modulators of other transcription factors and epigenetic effectors (1). They are also components of a number of important signaling pathways in embryonic development, such as the mitogen-activated protein kinase (MAPK), AKT1 (AKT) and Hedgehog pathways (2).

The deregulation of FOX proteins can change cell fate, which is closely related to developmental genetic diseases and malignancies and plays a key role in many cancers including leukemia. Based on this, FOX proteins provide potential targets for diagnosis and treatment of a multitude of human cancers. Accumulating evidence suggests that FOXM1 and FOXOs are correlated with various biological processes in leukemia development, such as leukemia initiation, progression and drug resistance after chemotherapy. This review highlights the complex regulatory mechanisms of FOXM1 and FOXOs in leukemia, their critical roles in the pathogenesis of leukemia and questions remaining to be addressed concerning these issues. This review also summarizes the clinical relevance of these FOX proteins and discusses their potential as therapeutic targets and prognostic markers in leukemia.

2. Aberrant expression of FOXM1 and its oncogenic roles in leukemia

FOXM1 is previously known as HFH-11, MPP-2, WIN and Trident. The locus of the human FOXM1 gene is situated at chromosome 12p13-3. It consists of 10 exons, including an exon Va (A1) and an exon VIIa (A2) which are alternatively spliced, producing 3 isoforms: FOXM1A, B and C (3). The FOXM1A isoform is transcriptionally inactive, whereas both FOXM1B and FOXM1C are transcriptionally active. FOXM1 is expressed in proliferating cells, but not in quiescent or terminally differentiated cells (3). FOXM1 stimulates expression of CCNA2 (cyclin A2), CCNB1 (cyclin B1) and CDC25B phosphatase as well as degrades CDK2 inhibitors CDKN1A (P21CIP1) and CDKN1B (P27KIP1), which is decisive for cell proliferation (3). FOXM1 regulates cell cycle progression and genomic stability by activating expression of many genes, such as AURKA (Aurora-A), AURKB (Aurora-B), PLK1, SKP2, CKS1BP7 (CKS1), BIRC5 (survivin) and CENPA, B, F isoforms (3). Upregulation of FOXM1 expression is found in different human carcinomas (1). FOXM1 is a pleiotropic player in tumors and its deregulation is associated with tumorigenesis and cancer progression. First, the involvement of FOXM1 in cancer initiation correlates with its roles in cell cycle progression and proliferation. Second, FOXM1 initiates angiogenesis by stimulating VEGFA expression in solid tumors (1). Third, epithelial-mesenchymal transition (EMT) induced by FOXM1 overexpression through activation of

CAV1 is related to the invasiveness and aggressiveness of cancer (1). Furthermore, upregulation of FOXM1 expression triggers cancer invasion and metastasis through activation of MMP2 and MMP9 expression (1). Finally, overexpression of FOXM1 enhances OCT4 transcription and induces stem cell phenotypes of cancer cells, leading to cancer progression and relapse (1). FOXM1 is regulated by various oncogenic signaling pathways and oncogenes or tumor-suppressor pathways and tumor suppressors in cancer (Table I). FOXM1 is a downstream effector of not only oncogenic KRAS (RAS)-MAPK1, 3 (MAPK), Sonic Hedgehog, NFKB1 (NF-κB) and EGFR signaling pathways, but also the TP53 (P53)-CDKN1A and CDKN2A (P16)-RB1 (pRB) tumor suppressor pathways in different carcinoma cells (3-10). Upstream oncogenes, such as E7, CCND1 (cyclin D1)/CDK4, CDK6 and NPM1 (nucleophosmin) and tumor suppressors, such as FOXO3, CDKN2A, CHEK2 (CHK2) interact with FOXM1 and mediate its expression (11-17). Emerging evidence has shown that microRNAs (miRNAs) such as miR-370, miR-134, miR-31 and miR-149 downregulate FOXM1 expression directly and their deregulation plays a part in cancer initiation and progression (Table I) (18-21).

Several lines of evidence that FOXM1 contributes to the pathogenesis of leukemia have been reported even though they are not as comprehensive as those of other cancers. FOXM1 is overexpressed in both AML cell lines and primary AML cells (22,23). In AML cells, FOXM1 is a key mediator of cell proliferation and governs cell cycle progression through modulation of the expression of cell cycle-related proteins (22). FOXM1 knockdown with FOXM1 siRNA was found to reduce cell proliferation and tumorigenicity by suppressing the expression of AURKB, BIRC5, CCNB1 and CDC25B and increasing the expression of CDKN1A and CDKN1B in AML cells (22). Previous studies have shown that overexpression of FOXM1 initiated by downregulation of tumor-suppressor miR-370 expression is involved in AML and CML development (23,24). FOXM1 was found to target MYC (c-myc), SKP2 and TERT (hTERT) as well as CDKN1B, and its aberrant expression is essential for the proliferation of AML cells (23). A recent study revealed that FOXM1 is a target of STAT3, and aberrant FOXM1 expression depends on constitutively activated STAT3, which is essential for the proliferation, survival and drug resistance of CML cells (25). Upregulated FOXM1 mediates multiple genes and signaling pathways involved in other significant cellular processes such as CCNB1, AURKA, CDC25B and SKP2 in cell cycle progression, BRCA1 and ATM signaling in DNA repair in CML cells, which correlates closely with leukemia development and progression (25). Overexpression of FOXM1 is associated with existence of FLT3-ITD receptors in AML cells (26). Up to 30% of AML patients have internal tandem duplications (ITD) within the FLT3 gene. FLT3-ITD receptors maintain constitutive tyrosine kinase activity even though no FLT3 ligand binds. It has been demonstrated that inhibition of FLT3-ITD by FLT3 receptor tyrosine kinase inhibitor AC220 hinders FOXM1 expression in MV4-11 AML cells (26). Taken together, the reported studies suggest that upregulation of FOXM1 is crucial for myeloid leukemia development and regulates many genes and cellular processes in myeloid leukemia (Fig. 1).

Table I. Important regulators interacting upstream of FOXM1 in cancer.

| Regulator	Effect	Identified cancer type(s)	Authorss (ref.)
Signaling pathways			
KRAS-MAPK1, 3	+	Osteosarcoma	Major et al (4)
Sonic Hedgehog	+	Basal cell carcinoma	Teh et al (5)
NFKB-1	+	Laryngeal squamous cell carcinoma	Penzo et al (6); Jiang et al (7)
EGFR	+	Breast cancer	Bektas et al (8)
TP53-CDKN1A	-	Hepatocellular carcinoma, colorectal carcinoma	Barsotti et al (9); Rovillain et al (10)
CDKN2A-RB1	-	Osteosarcoma	Barsotti et al (9); Rovillain et al (10)
Molecules			
E7	+	Cervical squamous carcinomas	Luscher-Firzlaff et al (11); McMurray et al (12)
CCND1/CDK4, 6	+	Melanoma, osteosarcoma	Anders et al (13)
NPM1	+	Osteosarcoma, breast cancer, pancreatic cancer	Bhat et al (14)
STAT3	+	Leukemia	Mencalha et al (25)
FOXO3	-	Breast cancer	McGovern et al (15)
CDKN2A	-	Hepatocellular carcinomas	Kalinichenko et al (16)
CHEK2	-	Osteosarcoma	Tan et al (17)
miR-370	-	Gastric cancer, leukemia	Feng et al (18); Zhang et al (23)
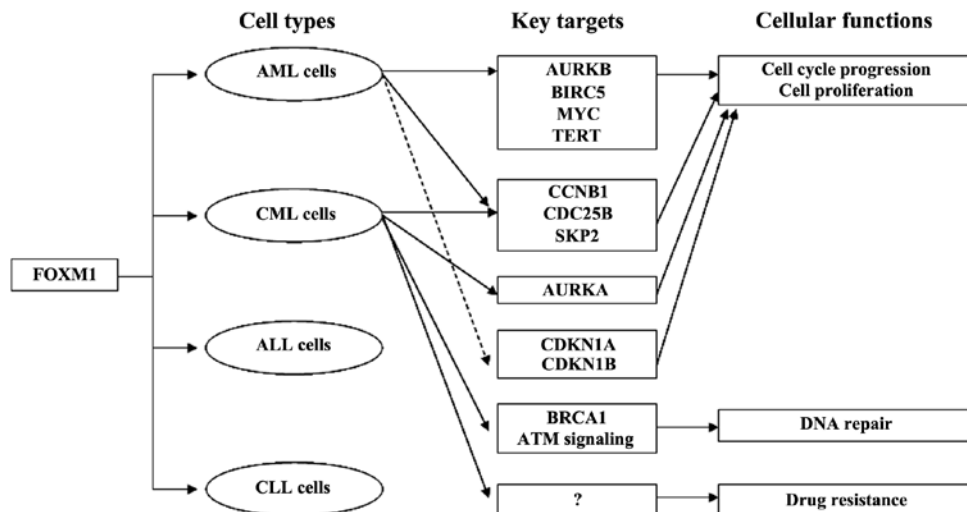
Figure 1. Schematic representation of key FOXM1 target genes and their cellular functions in different leukemic cells. Aberrant expression of FOXM1 contributes to leukemogenesis through modulating genes involved in cell cycle progression, cell proliferation, DNA repair and drug resistance. AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia. → stimulates; - - -→ inhibits.

3. Deregulation of FOXO transcription factors and the regulatory mechanisms in leukemia

FOXOs constitute one of the largest subgroups of forkhead family members. There are four members in this subfamily: FOXO1, FOXO3, FOXO4 and FOXO6. They govern a wide variety of cellular processes including cell cycle arrest, apoptosis, DNA damage repair, stress response, angiogenesis and metabolism. They activate or repress multiple genes involved in these cellular processes such as BAD and FASLG (FASL) in apoptosis, CDKN1A, CDKN1B and PLK1 in cell cycle progression, GADD45A in DNA damage repair, and CAT and SOD2 in oxidative stress (1,27). Post-translational modifications, such as acetylation, methylation and ubiquitination, are required for normal functioning of FOXOs (27). Accumulating data support that FOXOs are bona fide tumor suppressors. Inactivation of FOXO proteins has been discovered in a number of malignancies including breast cancer, prostate cancer, glioblastoma and leukemia (27). AKT1, IKBKB (IKK) and MAPK1 (ERK) are three commonly activated oncogenic kinases and target FOXO3 (FOXO3A) in human cancers (Table II) (28-30). Oncogenic kinases AKT1, IKBKB and MAPK1 phosphorylate

Table II. Important regulators interacting upstream of FOXO transcription factors in cancer.

| FOXO factor	Regulator	Effect	Identified cancer type	Authors (ref.)
FOXO3	Signaling pathways			
	PIK3CA-AKT1	-	Leukemia	Brunet et al (28)
	NFKB1-IKBKB	-	Breast cancer	Hu et al (29)
	MAPK1	-	Breast cancer	Yang et al (30)
	TGFB1-AKT1	+	Leukemia	Naka et al (51)
	MAPK8-JUN	+	Leukemia	Sykes et al (54)
	Molecules			
	miR-182	-	Lymphoblastic malignancies	Yang et al (32)
FOXO1	Signaling pathways			
	PIK3CA-AKT1	-	Leukemia	Hussain et al (47)
	Molecules			
	miR-9	-	Leukemia	Senyuk et al (34)
FOXO4	Signaling pathways			
	PIK3CA-AKT1	-	Leukemia	Oteiza et al (48)
	Molecules			
	tax	-	Leukemia	Oteiza et al (48)
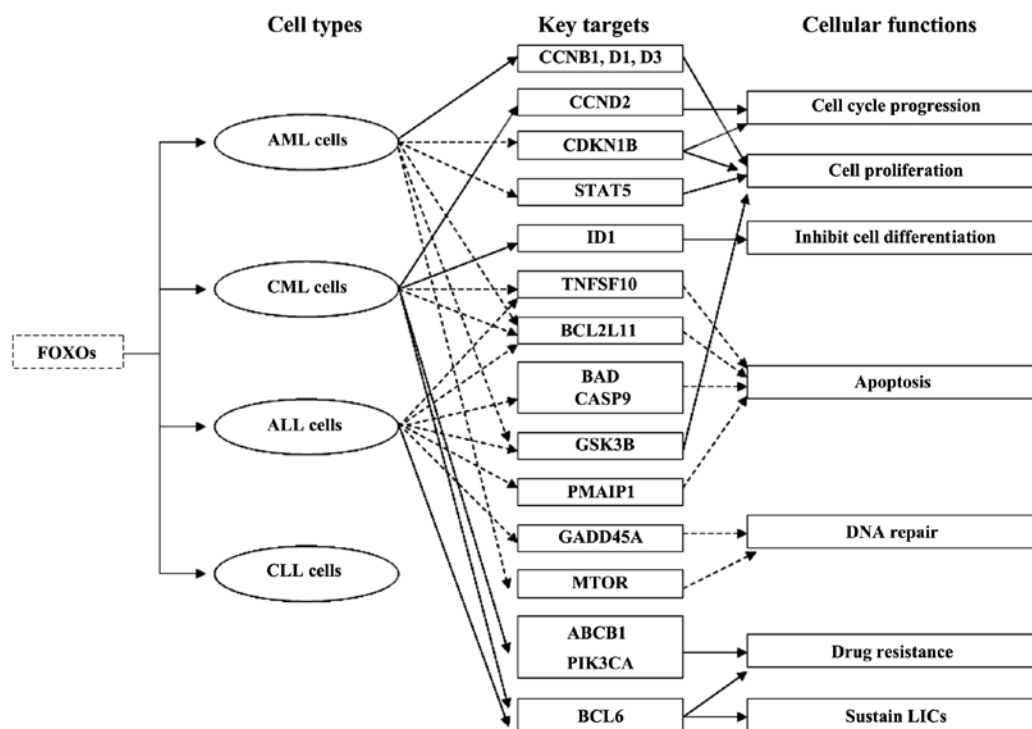
Figure 2. Schematic representation of key FOXO target genes and their cellular functions in different leukemic cells. Aberrant expression of FOXOs contributes to leukemogenesis through mediating genes involved in multiple cellular processes as presented. AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; LICs, leukemia-initiating cells. → stimulates; - - -▸ inhibits.

FOXO3 at different phosphorylation sites in response to external stimulation, resulting in FOXO3 nuclear exclusion, degradation and final transcriptional suppression. Additionally FOXOs are regulated by miRNAs, such as miR-155, miR-182, miR-224, miR-9 and miR-421, which is essential for many cellular processes in terms of cell survival, cell proliferation and tumorigenesis. Deregulation of these miRNAs is one of the underlying mechanisms of cancer development including leukemia development (Table II) (31-35).

Aberrant expression of FOXOs plays an important role in leukemogenesis (Fig. 2). At times chromosomal translocations in leukemia create FOXO fusion genes. The fusion proteins KMT2A (MLL)-FOXO3 and KMT2A-FOXO4 have been demonstrated to immortalize myeloid progenitors and induce

AML as the result of oncogenic activation of KMT2A by CR2 and CR3 which are transcriptional effector domains of FOXO3 and FOXO4 and subsequent KMT2A-mediated cellular transformation (36).

To date, several findings have concluded that the inhibition of FOXO3 is involved in the signaling cascade by which the BCR-ABL1 (ABL) fusion gene initiates oncogenic transformation in leukemic cells. A previous study showed that conditional inhibition of BCR-ABL1 by STI571 (imatinib, Gleevec) activated FOXO3 expression and downregulated CCND2 (cyclin D2) expression in BCR-ABL1-positive CML cells. This finding also suggests that BCR-ABL1 induces FOXO3 and BCL6 inactivation, CCND2 upregulation and cell cycle progression may be responsible for the oncogenic transformation of CML (37). Another study revealed that overexpression of ID1 (inhibitor of DNA binding 1) mediated by AKT1 activation/FOXO3 inhibition is required for leukemic transformation in BCR-ABL1-positive CML cells (38). Constitutively active ID1, which is a transcriptional target of FOXO3, inhibits cell differentiation and generates a differentiation block, leading to leukemic transformation of hematopoietic cells (38). The BCR-ABL1 oncoprotein induces leukemogenesis through stimulating PIK3CA (PI3K)-AKT1-FOXO3 signaling, which promotes cell proliferation and prevents apoptosis (39-41). Active PIK3CA-AKT1 signaling causes phosphorylation of FOXO3 and its retention in the cytoplasm, leading to inhibition of FOXO3 expression and downregulation of FOXO3 targeting cell cycle inhibitory genes and pro-apoptotic genes, such as TNFSF10 (TRAIL) and BCL2L11 (BIM) in BCR-ABL1-positive CML and acute lymphoblastic leukemia (ALL) (41). In addition, the proteasome-dependent degradation and suppression of FOXO3 protein, which results in inhibition of the FOXO3 targets, is responsible for evasion of apoptosis and leukemogenesis in BCR-ABL1-driven CML and ALL cells (41).

Inactivation of FOXO3 function mediated by oncogenic tyrosine kinase FLT3 also plays a part in leukemogenesis. Experiments performed in a Tet-On Ba/F3 cell line, which expresses FLT3-ITD depending on doxycycline, have shown that FLT3-ITD receptors inhibit apoptosis and promote cell proliferation through phosphorylation of FOXO3 and suppression of its target genes CDKN1B and BCL2L11 (42). These data suggest that FLT3-ITD expression induced suppression of FOXO3 and its target genes may be the underlying mechanism of oncogenic transformation in hematopoietic malignancies such as human AML (42). Furthermore, studies *in vitro* and *in vivo* as well as in samples from AML patients have revealed that constitutive AKT1 activation and FOXO3 inactivation induced by FLT3-ITD lead to cell proliferation and leukemic transformation of myeloid cells in AML (43).

FOXO3 suppression triggered by its upstream oncogenic signaling pathways is crucial for the uncontrolled proliferation and survival of leukemic cells. FOXO3 is known to be regulated by the PIK3CA-AKT1, MAPK1 (ERK-MAPK) and IKBKB signaling pathways in AML cells (44). More recently, an in-depth study revealed that the nuclear exclusion of FOXO3 and its inactivation are not due to the deregulation of the PIK3CA-AKT1 or the MAPK1 signaling pathway but the constitutive active IKBKB activity in AML cells (44). These findings indicate that rescuing FOXO3 activity by IKBKB inhibitors is necessary for AML therapy because of IKBKB-dependent FOXO3 regulation in AML.

In T-cell acute lymphoblastic leukemia (T-ALL) cells, PIK3CA-AKT1-FOXO1 signaling is constitutively active because of mutated PTEN phosphatase which is a tumor suppressor and negative modulator of the PIK3CA-AKT1 pathway (45-47). Mutations in PTEN phosphatase generate an anti-apoptotic effect and promote cell survival through activation of PIK3CA-AKT1-FOXO1 signaling. Specifically, active PIK3CA-AKT1-FOXO1 signaling causes phosphorylation of BAD (Bad), GSK3B (GSK3) and CASP9 (caspase-9) and prevents apoptosis, resulting in leukemogenesis (45-47).

FOXO4 inactivation contributes to adult T-cell leukemia (ATL) induced by human T-cell leukemia virus type 1 (HTLV-1). The HTLV-1 tax oncoprotein downregulates FOXO4 transcriptional activity and is a master regulator in HTLV-1 initiated oncogenic transformation of infected T cells (48). Tax oncoprotein activates the PIK3CA-AKT1 pathway, in turn stimulates FOXO4 phosphorylation and nuclear exclusion and mediates ubiquitination, proteasomal degradation and inhibition of FOXO4 and its target genes such as GADD45A in HTLV-1-transformed cells (48).

Although accumulating data highlight that FOXOs function as tumor suppressors in leukemia; paradoxically, FOXOs also have been shown to be important for maintenance of leukemia stem cells and responsible for drug resistance in leukemia. FOXO3 plays a dual role of sensitivity and resistance in response to chemotherapeutic drugs in leukemia. Following doxorubicin treatment, FOXO3 initially induces cell cycle arrest and apoptosis; thereafter, continued activation of FOXO3 leads to drug resistance by stimulating ABCB1 (MDR1) and PIK3CA expression in CML cells (49,50). Specifically, FOXO3 regulates PIK3CA catalytic subunit p110α directly, enhances PIK3CA/AKT1 activity, causes phosphorylation of FOXOs and their nuclear exclusion, thereby inhibiting or activating FOXO target genes that are important for cell proliferation, apoptosis and differentiation (50). These findings suggest that FOXO3 may be an ideal target with which to prevent MDR (multidrug resistance) in CML. FOXO3 has been confirmed to be required for maintenance of CML stem cells which trigger the recurrence of CML after tyrosine kinase inhibitor (TKI), imatinib therapy and the TGFB1 (TGF-β)-AKT1-FOXO3 pathway is not only essential for the survival of imatinib-resistant CML stem cells but also contributes to tumorigenicity of CML cells (51). Leukemia-initiating cells (LICs) are characterized by suppressed AKT1 phosphorylation and nuclear localization of FOXO3. TGFB1 regulates the AKT1-FOXO3 pathway and promotes nuclear localization of FOXO3 in LICs (51). Recently, another study demonstrated that FOXO3-BCL6-CDKN2A (ARF)/TP53 signaling pathways are involved in sustaining LICs after TKI treatment in CML and Philadelphia chromosome-positive (Ph+) ALL and prolonged treatment with a combination of AKT1-FOXO3 pathway inhibitor imatinib and BCL6 peptide inhibitor RI-BPI could eradicate LICs efficiently (52,53). Proto-oncogene BCL6 sustains leukemia stem cells and induces drug resistance by suppressing the CDKN2A-TP53 pathway after TKI treatment in leukemia driven by BCR-ABL1 fusion genes (52,53). Moreover, a recent study discovered that FOXOs play pivotal roles in the maintenance of LICs by preventing

their differentiation and apoptosis and are involved in leukemogenesis (54). The MAPK8 (JNK)-JUN (c-JUN) pathway stimulates FOXO nuclear localization and antagonizes the effect caused by FOXO inhibition in LICs in AML (54). Different from AML and CML cells, evasion of apoptosis and drug resistance are linked to both mutated tumor-suppressor CDKN2A (P16INK4A) and elevated AKT1 (PKB)-FOXO3 signaling, which decreases TNFSF10 and PMAIP1 (Noxa) expression in pediatric T-ALL cells (55).

4. Leukemia therapeutics targeting FOXM1 and FOXOs

Several lines of evidence suggest that chemical inhibitors targeting FOXM1 may be developed as novel antileukemic agents which may supplement present remedies. FOXM1 inhibitors/thiazole antibiotics siomycin A and thiostrepton, which are potent inhibitors of FOXM1 transcriptional activity and FOXM1-dependent transcription, efficiently inhibit cell growth and induce apoptosis in MV4-11, THP1, CEM, HL60 and U937 leukemia cell lines (26,56). Proteasome inhibitors MG115, MG132 and bortezomib have been demonstrated to induce apoptosis by inhibiting FOXM1 transcriptional activity and expression in the HL-60 leukemia cell line (57). Moreover, two phospha sugar derivatives, 2,3,4-tribromo-3-methyl-1-phenylphospholane 1-oxide (TMPP) and 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (DMPP) have been discovered to inhibit FOXM1 expression, inducing G2/M cell cycle blockage at low concentrations and apoptosis at high concentrations in leukemic cells (58). They are promising agents in targeted antileukemic therapy. Homoharringtonine (HHT), which is a traditional Chinese medicine used for AML and CML treatment, has been shown to upregulate the level of miR-370 directly and inhibit FOXM1 expression, inducing apoptosis in CML cells (24). Addition of miR-370 mimics enhanced the efficacy of HHT through suppressing FOXM1 expression (24). In addition, CDKN2A, an upstream tumor suppressor of FOXM1, has been confirmed to be successfully used as a therapeutic intervention target *in vitro* and *in vivo* in liver cancer (59). These data suggest that developing agents which target upstream interactive oncogenes, tumor suppressors and signaling pathways of FOXM1 may be effective approaches for leukemia therapy (Table I).

Imatinib, a regularly used antileukemic agent, has been confirmed to inhibit BCR-ABL1 by activating FOXO3 and inducing BCL2L11-dependent apoptosis in CML (40). Due to the potent tumor-suppressing function of FOXO3, drugs that activate FOXO3 and its downstream genes similar to imatinib are potential antileukemic agents. Proteasome inhibitor bortezomib (Velcade) was found to induce apoptosis by activating the expression of FOXO3 and its downstream pro-apoptotic genes TNFSF10 (TRAIL) and BCL2L11 in both imatinib-sensitive and imatinib-resistant leukemic cells from patients with BCR-ABL1-positive CML or ALL (41). Bortezomib has been proven to be a promising chemotherapeutic agent with which to treat BCR-ABL1-induced leukemia (41). Cell penetrating TAT-FOXO3 fusion proteins have been reported to induce apoptotic cell death in Jurkat, K562 leukemic cells and primary cells from chronic lymphocytic leukemia (CLL) patients (60). They are probably developed as effective antileukemic agents. FOXO3 activa-

tion has been shown to be essential for the efficacy of the alisertib/cytarabine combination therapy in AML (61). Either single agent or the combination of both agents induced expression of FOXO3 and its pro-apoptotic targets, CDKN1B and BCL2L11, therefore leading to apoptosis in AML cells (61).

Besides targeting FOXO3, activating other FOXO transcription factors may be valid for leukemia treatment. AKT1-FOXO1 signaling is constitutively active in T-ALL cells and suppressing the pathway by curcumin leads to cell growth inhibition by GSK3B inactivation (47). Curcumin inhibits cell proliferation and promotes apoptosis through dephosphorylation of FOXO1 and successive activation of apoptotic signaling in T-ALL cells (47).

5. Additional clinical relevance of FOXM1 and FOXOs in leukemia

Upregulation of FOXM1 is linked to FLT3-ITD expression and adverse prognosis in AML (26). Inhibition of FLT3-ITD decreases FOXM1 expression and stimulation of FLT3 by the FLT3 ligand increases FOXM1 expression in AML cells, suggesting that FOXM1 could be a suitable prognostic marker for AML patients (26). In addition, it has been reported that FOXM1 target genes, which mediate several cellular processes including cell cycle, differentiation, ageing, genomic stability, epigenetic and stem cell renewal, can be used to diagnose and evaluate aggressiveness of early squamous carcinoma (62). These data suggest that FOXM1 and its target genes are potential biomarkers for leukemia diagnosis and prognosis.

Phospho-FOXO1 is detected in most AML patients because of phosphorylation of AKT1 signaling (63). The presence of phospho-FOXO1 was found to be correlated with reduced survival and an unfavorable outcome in AML patients, suggesting that phospho-FOXO1 is a valuable molecular marker for AML prognosis (63). High levels of phosphorylation of FOXO3 have also been found in AML patients and were confirmed to be an adverse prognostic factor in AML, which is relevant to increased proliferation, resistance to therapy and reduced survival (64). Higher levels of phospho-FOXO3 increased levels of CCNB1, D1 and D3, pGSK3B, pMTOR, and pSTAT5 and promoted cell proliferation, which was associated with higher WBCs, percent marrow and blood blasts in AML patients (64). Taken together, phospho-FOXOs are useful indicators with which to estimate the prognosis of AML patients.

Clinical evidence has shown that FOXO3 is an adequate theranostic marker for treatment with bortezomib in Ph+ ALL as FOXO3 is a key mediator in the pathogenesis of the disease and FOXO3 expression is suppressed after treatment with bortezomib (65). Moreover, comparing human bone marrow samples of Ph+ ALL, Ph- ALL and normal controls has revealed that FOXO3 downregulation is specific to Ph+ ALL (65).

6. Conclusion and future perspectives

Deregulation of FOXM1 and FOXOs is involved in leukemogenesis and leukemia development. The collective data support that overexpressed FOXM1 is a key player in cell proliferation, cell survival, cell cycle progression, drug resistance and DNA repair in leukemic cells, but they are not perfect answers to the

pathogenesis of leukemia in contrast to studies conducted in other cancers. The molecular mechanisms by which FOXM1 governs leukemia initiation, invasion, drug resistance and other cellular processes require further clarification. Moreover, the significance of FOXM1 in other types of leukemia such as ALL and CLL development is unclear and remains to be determined. In addition, it will be interesting and worthwhile to identify additional miRNAs which target FOXM1 and mediate its expression in leukemic cells. Targeting FOXM1 with therapeutic inhibitors may be an effective strategy for leukemia therapy. In addition, FOXM1 could be a valuable biomarker for leukemia diagnosis and prognosis due to its aberrant expression and cellular functions in leukemic cells, but further investigations needs to be carried out. The abnormal expression of FOXOs plays a prominent part in the proliferation and survival of leukemic cells and their evasion of apoptosis. Activation of FOXOs is responsible for not only the initial cytotoxic response to some antileukemic drugs but also subsequent resistance in leukemia. FOXO3-enriched leukemia stem cells and induced MDR genes contribute to the acquisition of drug resistance in leukemia. Developing therapeutic agents that restore the function of tumor-suppressor FOXOs may be rational and well suitable for leukemia treatment. But while developing therapeutic agents targeting FOXOs, we should consider the dual effects of FOXOs in response to chemotherapy. Additionally, FOXOs are potential diagnostic and prognostic tools for leukemia in the clinic.

Overall, therapeutic intervention of the upstream interactive oncogenes and tumor suppressors of FOXM1 and FOXOs including miRNAs and oncogenic and tumor-suppressor signaling pathways converging on FOXM1 and FOXOs as well as the FOX proteins themselves in leukemia may be reasonable strategies with which to treat the disease (Tables I and II). Therefore, identifying novel regulatory signaling pathways and molecules interacting upstream of FOXM1 and FOXOs in leukemic cells is critically important but very challenging.

Finally, it will be of value to clarify how aberrant expression of FOXM1 and FOXOs relates to various chromosomal translocations in leukemia, which will provide novel insights into leukemia pathogenesis, and may facilitate the development of novel therapeutics and prognostic markers.

Acknowledgements

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