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REVIEW ARTICLE

The Roles of FOXO3 and c-Myc as key regulatory genes in leukaemia and myelodysplastic syndrome

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Abstract

The human leukaemia develops with abnormal increase of blast cells in the bone marrow. Leukaemia is caused by genetic aberrations which activates proto-oncogenes and inactivates tumor-suppressor genes and eventually leads to leukemogenesis. Myelodysplastic syndrome is a preleukemic state which shares similar symptoms and causative factors as leukaemia. FOXO3 and c-Myc have been increasingly recognized as key regulatory genes involved in the initiation and development of leukaemia and myelodysplastic syndromes. Their roles in these diseases is being investigated and findings thus far has indicated that FOXO3 acts as a tumor suppressor while c-Myc has been identified as a proto-oncogene. Currently published literature indicate that there are limited research on the correlation between FOXO3 and c-Myc especially in leukaemia and myelodysplastic syndrome. This review will focus on the key regulatory roles of FOXO3 and c-Myc in leukaemia and myelodysplastic syndrome.

Keywords: Leukaemia, myelodysplastic syndrome, FOXO3, c-Myc

Introduction

Leukaemia is the cancer of the bone marrow or blood with abnormal increase in immature leukocytes (blasts). Leukaemia mainly results from genetic aberrations that cause activation of proto-oncogenes and inactivation of tumor-suppressor genes eventually leading to leukemogenesis (Zhu, 2014). Leukaemia is classified into four major types: acute myeloid leukaemia, acute lymphocytic leukaemia chronic myeloid leukaemia, and chronic lymphocytic leukaemia. Acute myeloid leukaemia (AML) is the rapid and excessive proliferation of myeloid progenitor cells which leads to accumulation of blasts in the bone marrow. Previously, AML has been classified by World Health Organization (WHO) using FAB (French-American-British) classification system (M0 to M7) according to the differentiation type and stage (Delgado and León, 2010). Later on in conjunction with clinical advances and new discoveries, WHO proposed a whole new classification system for AML that incorporates genetic, morphology and immunophenotypic information in addition to clinical presentations. There are about six major categorization that are: AML with recurrent genetic abnormalities, AML with myelodysplasia-related features, therapy-related AML, AML not otherwise specified, myeloid sarcoma and myeloid proliferation related to Down syndrome (Arber et al., 2016). Acute lymphocytic leukaemia (ALL) is a heterogeneous disease with clonal expansion of lymphoblasts or the excessive proliferation of lymphoid progenitor cells from either B or T cell lineage (Delgado and León, 2010; Shah et al., 2014). Chronic myeloid leukaemia (CML), on the other hand, is the excess

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proliferation of myeloid cells but it is slow developing and has lesser blasts in the bone marrow as compared to AML. CML is characterized by three phases: chronic, accelerated and blast crisis (Shah et al., 2014). Meanwhile, chronic lymphocytic leukaemia (CLL) is a slow progressing leukaemia in adults, with excessive proliferation and accumulation of mature lymphocytes in the bone marrow, blood and lymph nodes (Shah et al., 2014). Myelodysplastic syndrome (MDS), previously known as pre-leukaemia, is a clonal haematopoietic stem cell disorder characterized by impaired peripheral blood cell production (cytopenias) and hypercellular, dysplastic-appearing bone marrow. It has substantial risk of progression to acute myeloid leukaemia (AML) thus results in increased mortality (Nimer, 2008). It is a well-known fact that leukemias and MDS is frequently caused by suppression of tumour suppressor genes and overexpression of oncogenes. Therefore, this review would focus on two important genes; FOXO3 and c-Myc. Both genes have been shown to be important for leukemogenesis (Zhu, 2014). FOXO3 is a well-known tumour suppressor gene whereas c-Myc is widely known as a proto-oncogene.

FOXO3

Forkhead box (FOX) proteins are a superfamily of transcriptional factors encoding a winged-helix DNA binding motif and the forkhead domain (Obsil and Obsilova, 2008). FOXO factors belongs to class O of this superfamily and it is the largest mammalian subgroup which consist of four members (FOXO1, FOXO3, FOXO4 and FOXO6) (Fu and Tindall, 2008). FOXOs composed of four distinct functional motifs including a forkhead, nuclear localization sequence, nuclear export sequence and transactivation domains (Wang, Zhou and Graves, 2014). FOXO proteins function predominantly as transcription factors in the nucleus and bind as monomers to their cognate DNA-targeting sequences (Fu and Tindall, 2008). FOXOs play critical role in diverse cellular processes such as cell cycle arrest, apoptosis, autophagy, DNA damage repair, stress resistance, angiogenesis, inflammation, differentiation and metabolism by transcriptionally activating and inhibiting downstream target genes (Zhu, 2014; Wang, Zhou and Graves, 2014).

Regulation and interactions of FOXO3 gene

FOXO3 (FOXO3a), a protein that interacts with numerous signal transduction pathways, is increasing being recognised as a master signalling regulator which control various physiological and pathological processes, including cancer protection (Fu and Tindall, 2008). FOXO3 suppress tumour growth in carcinogenesis and restoring of FOXO3 activity is known to promote tumour cell death (Wang, Zhou and Graves, 2014). Regulation of the subcellular localization and transcriptional activity of FOXOs require post-translational modifications, such as phosphorylation, acetylation, methylation, and ubiquitination (Fu and Tindall, 2008; Yang and Hung, 2009). Its function could be lost by either diminished expression or inactivation by phosphorylation (Kornblau et al., 2010). Three commonly activated oncogenic kinases which target FOXO3 in human cancers are AKT1, IKBKB (IKK) and MAPK1(ERK), and they phosphorylate FOXO3 at different phosphorylation sites in response to external stimuli such as epidermal growth factors receptors (EGFR), insulin, insulin-like growth factor, neurotrophins, nutrients, cytokines and oxidative stress (Zhu,2014; Yang and Hung, 2009). These stimuli control FOXO protein levels, subcellular localization, DNA-binding and transcriptional activity (Calnan and Brunet, 2008).

FOXOs are mostly localized in the nucleus in the absence of insulin/growth factors. Insulin and growth factors will activate the PI3K–Akt/SGK pathway and trigger the phosphorylation of FOXOs in the nucleus, the binding of the chaperone protein 14-3-3 to FOXO and the release of FOXO from their DNA-binding sites (Calnan and Brunet, 2008). AKT causes phosphorylation on threonine 24, serine 256 or serine 318 and inhibits FOXO3a activity (Arden, 2004). The binding of 14-3-3 may lead to the exposure of nuclear export sequence (NES) and facilitate the interaction between FOXO and Ran/Crm1 at the nuclear pore, allowing active export of FOXO to the cytoplasm. The binding of 14-3-3 protein to FOXO factors also prevent FOXO re-entry into the nucleus by blocking the nuclear localization signal (Calnan and Brunet, 2008; Brunet el al., 2002). In the cytoplasm, where ubiquitination is induced, phosphorylated FOXO is degraded by proteasome-dependent degradation and transcriptional activity is suppressed which then inhibits transcription of the genes that promote

apoptosis (Bim, FasL and TRAIL) and cell cycle arrest ($p27^{Kip1}$, $p21^{Cip1}$, and c-Myc) (Kornblau et al., 2010; Calnan and Brunet, 2008).

Acetylation is similar to phosphorylation in increasing and decreasing FOXO transcriptional activity. Histone acetyltransferase and histone deacetylases control the effect of acetylation on FOXOs. In the presence of stress stimuli, FOXO3 is acetylated at K242, K259, K271, K290, and K569 (Wang, Zhou and Graves, 2014). The expression of proapoptotic genes (*Bim, TRAIL, FasL*) are favourable at more highly acetylated forms of FOXO3 while expression of antioxidant and cytoprotective genes are favourable at more deacetylated forms. Acetylation exert inhibitory effects on FOXO transcriptional activity by reducing the DNA binding activity while deacetylation enhances the binding activity (Fu and Tindall, 2008; Wang, Zhou and Graves, 2014).

The role of FOXO3 in AML

FOXO3 genes are found at chromosomal breakpoints in human soft-tissue tumours and leukaemia, specifically at the t(6;11)(q21;q23) chromosomal translocation from an AML patient (Kornblau et al. 2010). In AML cells, FOXO3 is regulated by PIK3CA-AKT1, MAPK1 (ERK-MAPK) and IKBKB signalling pathways and deregulation of the oncogenic kinases such as AKT, ERK and IKK are often found in primary blast cells and leads to uncontrolled cell growth and survival (Chapuis et al., 2010). In addition, studies have also shown that the constant inactive state of FOXO3 is not due to the deregulation of the PIK3CA-AKT1 or the MAPK1 signalling pathway but rather the IKK activity which maintains FOXO3 in the cytoplasm that causes proliferation and survival of AML cells (Zhu, 2014; Chapuis et al., 2010).

FOXO3 deregulation contributes to the pathogenesis of AML through various molecular pathways involving crucial genes. Scheijen et al. (2004) suggested that a common gene mutation in AML, Fms-related tyrosine kinase 3 internal tandem duplication (FLT3-ITD) expression induces suppression of FOXO3 by phosphorylation and suppression of its target genes CDKN1B and BCL2L11 via dinhibition of apoptosis and induced cell proliferation, which may be the underlying mechanism of oncogenic transformation in hematopoietic malignancies such as human AML. In addition, *in vitro* and *in vivo* studies showed that cell proliferation and leukemic transformation of myeloid cells in AML by FLT3-ITD is due to activation of AKT (PKB) which phosphorylates and inhibits FOXO transcriptional factors. AKT phosphorylation causes transformation of 32D myeloid progenitor cells *in vitro* and promote development of leukaemia-like myeloid disease *in vivo* (Brandts et al. 2005).

Phosphorylation of FOXO3 is an adverse prognostic factor in AML that is associated with increased proliferation, resistance to therapy and shorter survival (Kornblau et al. 2010). Enhanced phosphoinositide 3-kinase PI3K/AKT activity increases expression of nuclear FOXO3 which is associated with increased drug resistance in leukaemic cells (Hui et al., 2008). Other than that, a study found that high FOXO3 mRNA levels is correlated with inferior survival in AML patients. Levels of CCNB1, CCND1, CCND3, pGSK3B, pMTOR and pSTAT5 that are increased by higher levels of phospho-FOXO3 in AML patient promotes cell proliferation, which was associated with higher whole blood counts increased percentage of marrow and blood blast (Figure 1). Patient noted to experience higher rate of primary resistance and shorter remission durations due to surge of FOXO3 phosphorylation. (Kornblau et al., 2010). Proliferation is increased when FOXO3 is inactivated by phosphorylation and makes leukaemic blast survive therapy, leading to higher rates of resistance and more rapid leukemic recurrenceInterestingly, , high levels of unphosphorylated FOXO3 maintained stem cells in a resting state and caused the emergence of resistant stem cells, enabling them to survive and repopulate the marrow with leukemic cells (Kornblau et al., 2010).

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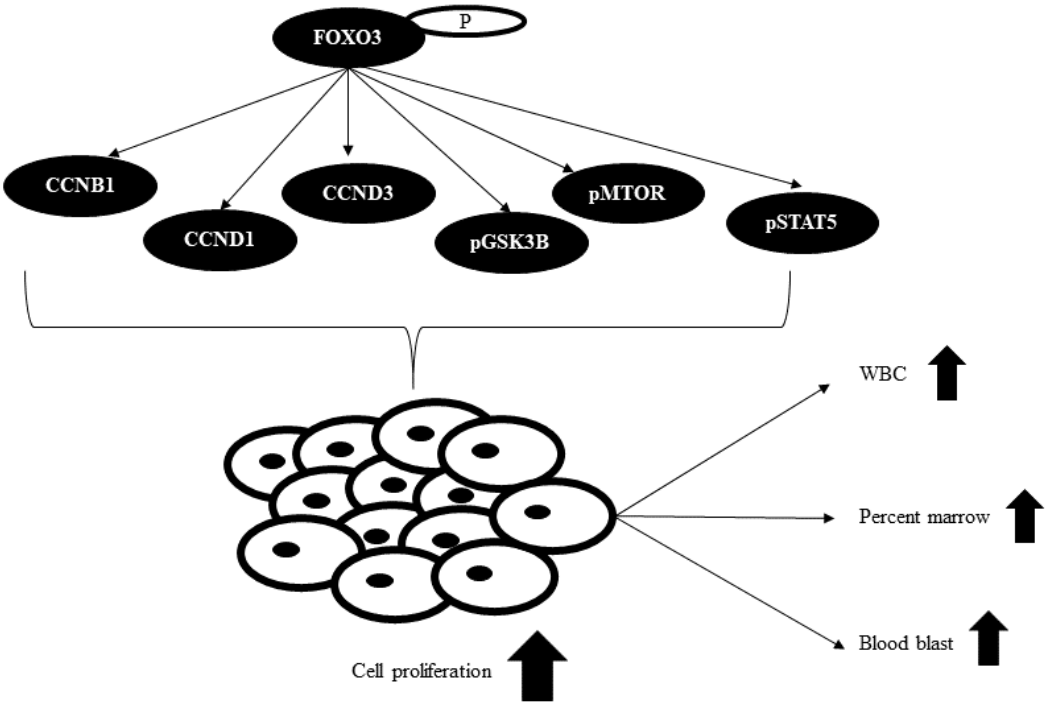

Figure 1. Interaction between FOXO3 and cell proliferation in AML.
CCNB1: Cyclin B1; CCND1: Cyclin D1; CCND3: Cyclin D3; pGSK3B: phospho-Glycogen synthase kinase 3 beta; pMTOR: phospho-Mammalian Target of Rapamycin; pSTAT5: phospho-Signal transducer and activator of transcription 5

MicroRNA (miRNA) are small non-coding RNA molecules that involves in regulation process by targeting mRNA for cleavage or translational repression (Gebert and MacRae, 2019). Recently, it has come to light that miRNAs contribute to AML development via FOXO3 modulation. For example, miR-155 was found to negatively regulate FOXO3 expression in AML cell line that ultimately results in increased cell proliferation and apoptosis inhibition (Zhang et al., 2016). Another study has found that oncogenic miR-183-5p negatively regulates FOXO3 via indirect effect in AML cell lines which leads to increase cell proliferation (Zheng et al., 2019). A study found that KP372-1, an AKT inhibitor can inhibit AKT activity by activating FOXO3 and inducing apoptosis in AML (Yang and Hung, 2009).

The role of FOXO3 in ALL

Of all the FOXO family proteins, FOXO3 was found to be abundantly expressed in Notch1-mutated T-ALL cell which includes BE-13, DND-41 and RPMI-8402. Western blot analysis has shown that after one hour of treatment with BMS-345541, an IKK inhibitor, FOXO3 nuclear translocation takes place and increases in the expression level of $p21^{Cip1}$ in these calls (Buontempo et al., 2012). Butein induces nuclear localization of FOXO3 which promotes the FOXO3 binding ability to $p27^{Kip1}$ promoter and in return, enhance the expression of $p27^{Kip1}$. For a stable FOXO3–$p27^{Kip1}$ complex, C-terminal region of FOXO3 DNA binding domain plays a significant role (Tang et al., 2016). In T-ALL cells, FOXO1 and FOXO3 are downstream targets of IL-7–mediated PI3K–Akt pathway. Thus, suggestion have been made that IL-7 may be responsible in downregulating $p27^{Kip1}$ by inactivation of FOXO3 via PI3K–Akt pathway (Barata et al., 2004).

Paediatric ALL cases shows FOXO3 gene was observed to be frequently deleted when compared between myeloid origin cell line and healthy bone marrow. The expression level of FOXO3 in ALL cell line such as Nalm6, Ball, ReH and Jurkat were lower with possible reason on gDNA of FOXO3 promoter hypermethylation in B-ALL cell line as compared to normal gDNA (Okamoto et al. 2010). In the Han Chinese population, C to T mutation of rs4946936 polymorphism located in the 3'UTR of FOXO3 has been identified as a risk

factor of childhood ALL (Wang et al. 2014). FOXO3 induces apoptosis in childhood T-ALL via caspase dependent manner with TRAIL and Noxa being important mediators (Ausserlechner et al., 2013).

Experiments in SLP-65-deficient pre-B cell line demonstrated the role of FOXO3 in inducing kappa light chain expression. It has been suggested that FOXO3 is necessary for normal B cell development. Precursor-B-cell receptor (pre-BCR) expression plays a key role in activating PKB which in return phosphorylates FOXO3, leading to its effects on light chain recombination. PKB was also found to be responsible in phosphorylating FOXO1 (Herzog et al., 2008).

Dexamethasone treatment of sensitive xenografts isolated from primary biopsy specimens of B-cell precursor ALL patients demonstrated that FOXO3 is activated in response to dexamethasone treatment and further support the mechanism of FOXO3 in activating Bim transcriptions (Bachmann et al., 2010; Gilley, Coffer and Ham, 2003; Jing et al., 2005). Another study has also determined that BIBR1532 (a telomerase inhibitor) along with doxorubicin enhance the expression level of FOXO3 and $p21^{Cip1}$ as well as increases the G1 phase cell population in Nalm-6 pre-B ALL cells (Bashash et al. 2013).

The role of FOXO3 in CML

Inhibition of FOXO function has played an important function in hematological malignancies such as leukaemias. The initiation of oncogenic transformation in leukemic cells by the signalling cascade of *BCR-ABL1* (ABL) fusion gene involves the inhibition of FOXO3 (Zhu, 2014). Leukemogenesis is induced by BCR-ABL1 oncoprotein through stimulating PIK3CA (PI3K)-AKT1-FOXO3 signalling which promotes cell proliferation and prevents apoptosis (Jagani et al., 2009; Zhu, 2014). FOXO3 activity was inhibited by BCR-ABL transformation by sustaining PI3-K-dependent constitutive phosphorylation and cytoplasmic retention of FOXO3 (Ghaffari et al., 2003; Komatsu et al., 2003). In both BCR-ABL-transformed cells which include Mo7e-p210 and BaF3-p210 as well as primary CML CD34+ cells, the activation of PI3-K/Akt pathway by BCR-ABL results in the upregulation of Skp2 transcriptional activity which probably causes leukemogenesis through increased proteasomal degradation and FOXO protein levels downregulation (Andreu et al., 2005). Suppression of downstream pro-apoptotic target TRAIL is observed in CML as a result from the inhibition of FOXO3 by BCR-ABL where the inhibition plays a role in controlling BCR-ABL-inducing transformation as expression of an actively performing FOXO3 triple mutant in BCR-ABL-transformed cells overrides the growth factor-independent survival and leads to apoptosis (Essafi et al., 2005; Ghaffari et al., 2003,). In a BCR-ABL-transformed cells present with FOXO3 dependent manner have been observed to suppress the BH3-only pro-apoptotic protein BIM, and apoptosis induced by imatinib in BCR-ABL-transformed cell lines have been contributed by FOXO3-mediated regulation of BIM (Essafi et al., 2005). In essence, inhibition of FOXO3 in BCR-ABL-transformed cells causes suppression of genes involved in the regulation of apoptotic program and cell cycle such as cyclin D2, eventually leading to tumorigenesis (De Mattos et al., 2004). Further evaluation on the inhibition of FOXO3 triggered by BCR-ABL has revealed that besides promoting phosphorylation at Akt-dependent sites, BCR-ABL also enhances the suppression of FOXO3 protein expression in a proteasome-dependent manner. FOXO3 function was restored by clinically relevant proteasome inhibitor bortezomib (VELCADE, PS-341) and apoptosis was induced not only in imatinib-sensitive, but also in imatinib-resistant (T315I) BCRABL- transformed patient cells (Jagani, Singh and Khosravi-Far, 2008).

The role of FOXO3 in CLL

Studies have supported the inactivation of FOXO3 as a key mediator in B-CLL resistance to apoptosis through homeostatic chemokines which can serve as a therapeutic target in this hematopoietic malignancy (Ticchioni et al. 2007). Prevention of FOXO3 nuclear translocation can be achieved through phosphorylation by Akt and sequestrated by 14-3-3 proteins in the cytosol in the presence of survival factors leading to the activation of target genes (Birkencamp and Coffer, 2003; Burgering and Medema, 2003). Furthermore, in the study, FOXO3 was proven to be constitutively phosphorylated on Threonine 32, a major Akt binding site of FOXO3 in B-CLL cells isolated from patients. In addition, this study also revealed that further phosphorylation of FOXO3 on Thr32 can occur through homeostatic chemokines treatment of B-CLL using CXCL 12. Exogenous

CXCL12 treatment on B-CLL cells was found to abrogate FOXO3 nuclear localization by enhancing its interaction with 14-3-3 protein and thus inhibiting the activation of proapoptotic genes (Ticchioni et al. 2007). Regulation of FOXO3 and dephosphorylation of Akt is a result of the inhibition of PI3K by NVP-BKM120 where the FOXO3 is one of the important effectors downstream of Akt (Fu and Tindall, 2008). FOXO3 can be activated by decreasing its phosphorylation and increasing its nuclear content, which causes the upregulation of genes involved in either apoptosis or cell cycle arrest in different types of cells such as the malignant cells of CLL (Brunet et al., 1999; Yang and Hung, 2009) . FOXO3 is an important regulator of Bim expression. Alterations in the Akt-FOXO3 axis have been suggested to affect Bim expression in several models where findings have revealed that plasmid based overexpression of FOXO3 in CLL cells reduced their survival and triggered the expression of Bim and p27 (Dijkers et al., 2000; Gilley, Coffer and Ham, 2003; Stahl et al. 2002). In addition, FOXO proteins are reported as important regulators of primary lymphocyte proliferation as activation of FOXO proteins led to cell-cycle arrest by increasing the expression of the cell-cycle inhibitor $p27^{Kip1}$ (Essafi et al., 2011). Furthermore, Akt-dependent phosphorylation and inactivation of FOXO3 was found to be the cause for spontaneous and chemokine-induced resistance to cell death in CLL cells (Ticchioni et al., 2007). It was also shown in a study by Essafi et al. (2011) that cell penetrating TAT-FOXO3 fusion proteins can induced apoptotic cell death in Jurkat, K562 leukemic cells, and primary cells from chronic lymphocytic leukaemia (CLL) patients.

The role of FOXO3 in MDS

Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem cell disorder which is characterised by dysregulated haematopoiesis that causes various cytopenias including thrombocytopenia, neutropenia and anaemia. MDS can frequently progress into acute myeloid leukaemia (Fenaux, 2004; Gilliland, Jordan and Felix, 2004). A study demonstrated that FOXO3 was predominantly hyper-phosphorylated in SKM-1 cells thus indicating that FOXO3 exist primarily in an inactive form in MDS. The study also showed that silencing of FOXO3 expression impaired DAC-induced cellular differentiation, apoptosis, and cell cycle arrest which was attributed mostly to down regulation of CDKN1B, CDKN1A and BCL2L11 genes (Zeng et al., 2017). A recent study has identified that pathogenesis of MDS could be contributed by promoter methylation of FOXO3. It was found that the greater risk subtypes of MDS exhibits higher level promoter methylation of FOXO3, indicating the use of FOXO3 as a prognostic factor in MDS (Sharifi et al., 2020).

c-Myc

Human MYC (c-Myc) is an oncogene that is located specifically at 4.5 Mb region of chromosome 8q24. c-Myc also behaves as a transcription factor that binds DNA and regulates genes expression involved in various cellular processes including proliferation, apoptosis, metabolic pathways, cell adhesion and angiogenesis (Dang, 2012). Studies discovered that human c-Myc, an oncogene, that is located, specifically 4.5 Mb region of chromosome 8q24 (Dalla-Favera et al., 1982; Poddighe et al., 2014). c-Myc is responsible in coding a basic helix-loop-helix leucine zipper transcription factor that dimerizes with Max and regulates various cellular function via transcription of downstream target genes (Dang, 1999; Dang et al., 2006). Its oncogene activation by viral promoter, chromosomal rearrangement, somatic mutation and gene amplification often leads to neoplastic transformation and malignancy (Dalla-Favera et al., 1982; Xiang et al., 2015). c-Myc ectopic expression has been found to inhibit cell differentiation in various cell lines and primary cells, and about half of them occurred in hematopoietic cell lines (Delgado and León, 2010). However, another study found that c-Myc proto-oncogene could lead to transformation and cell proliferation by activation of cyclin D2 promoter, at the same time c-Myc could induced apoptosis mediated by nuclear respiratory factor 1 (NRF-1) and Arf-p53 pathway. Forced expression of c-Myc in most primary cell types resulted in growth arrest or apoptosis (Luo et al., 2005).

The role of c-Myc in AML

Delgado and Leon (2010) reported that c-Myc translocation and amplification are uncommon in AML as compared to lymphoid malignancies as c-Myc over expression commonly occurs as a result of other recurrent oncogenic events (BCR-ABL,

RUNX1T1-RUNX1, PML-RARA and ZBTB16-RARA fusion genes) or somatic mutations (JAK2 and FLT3) in AML. c-Myc -Myc expression is induced by at least three of the fusion genes, thus suggesting that c-Myc is a downstream target of these oncogenes.

C-Myc amplification in double minutes (dmins) or homogenously staining regions (HSR) is often part of a complex karyotype in AML (Paulsson et al., 2003). Extrachromosomal amplification of the proto-oncogene c-Myc in dmin has been found in several cases of AML especially in FAB subtype M2 as c-Myc proto-oncogene is the most frequent amplified gene in AML cases (Dang, 1999). It was also found that extrachromosomal amplification of c-yMc proto-oncogene is related to the loss of chromosome 8 (Bruckert et al., 2000). Study from Bruckert et al. (2000) showed that the mechanism of c-Myc amplification in dmins or HSRs in AML is due to excision from an intact chromosome 8q24, circulation and amplification by mutual recombination. Amplification of c-Myc causes an overexpression of c-Myc protein which is a nuclear transcription factor and leads to aggressive growth and poor prognosis. Studies have also found that rapid development of AML in mice can be induced by ectopic overexpression of c-Myc (Luo et al., 2005). Previous studies have also proven that overexpression of c-Myc gene in myelogenous leukaemia cell lines results in rapid cell proliferation, cell cycle exit inhibition and differentiation termination (Wolff and Perkins, 2012).

However, studies in cell culture and animal model system also found that c-Myc overexpression alone is not enough to induce cell transformation or tumour development as further accompanying mutations are required (Cole, 1986; Bruckert et al., 2000). Therefore, AML cases with complex aberrant karyotypes may be due to c-Myc amplification with an addition of other oncogene activation and/or loss of tumor suppressor gene (Bruckert et al., 2000). Patients with normal karyotype and numerous c-Myc dimers might have a better response to chemotherapy with better prognosis compared to those with complex karyotype and few c-Myc dimers (Li, 1983). The combination of extrachromosomal c-Myc amplification and complex chromosomal aberrations will result in rapid disease progression and short survival time. Thus, the sole occurrence of dmin in AML especially with extra chromosomal c-Myc amplification is not an indication of poor prognosis and reduced responsiveness to chemotherapy. Therefore, more studies must be done to confirm this (Bruckert et al.,2000).

In addition, a study by Pan et al (2014) found that high expression of c-Myc is one of the characteristics in drug-resistant leukaemia cells. The study also showed that c-Myc up-regulation in leukaemic cells promoted colony formation ability and maintains poor differentiation by suppression of C/EBPβ thus resulting in drug resistance. Interestingly overexpression of C/EBPβ was found to reverse c-Myc induced drug resistance. Studies using shRNA or specific inhibitor towards c-Myc resulted in down-regulation of c-Myc, abrogation of colony forming capacity, promotion of cellular differentiation and up-regulation of C/EBPβ in primary AML cells (Pan et al., 2014).

The role of c-Myc in ALL

Studies have shown that c-Myc translocation t(8;14), t(8;22) and t(2;8) is present in 5% of adult ALL cases and 2- 5% of paediatric ALL cases (Faderl et al., 2010). Furthermore, more than 50% of human T-cell acute lymphoblastic leukaemia (T-ALL) have mutations in Notch1 (Grabher, von Boehmer and Look, 2006) which comes as no surprise as c-Myc is a direct downstream target of Notch1, a known regulator of quiescence in stem and progenitor populations, which contributes to the growth of T-ALL cells (Roderick et al. 2014; Weng et al. 2006). Pro-growth effect of activated Notch1 was disrupted by c-Myc inhibitors and enforced expression of c-Myc was found to rescue multiple Notch-dependent T-ALL cell lines from Notch withdrawal (Weng et al., 2006). Small hairpin RNA or pharmacologic approaches to suppress c-Myc was found to prevent leukaemia initiation in murine models by eliminating leukaemia-initiating cell (LIC) activity. The growth of treatment-resistant primary T-ALL patient samples *in vitro* was prevented by c-Myc inhibition. This study also found that treatment using the BET bromodomain BRD4 inhibitor JQ1 with its anti-LIC activity in mice, reduced c-Myc expression and inhibited the growth of relapsed and induction failure (IF) *in vivo* (Roderick et al. 2014).

HDAC7 induced apoptosis through p53 pathway activation and influenced the expression of

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apoptotic genes including CD44, FAS, ATM, TP53BP2, CD40 and BIRC3 and induces HDAC7 expression also resulted in down regulation of c-Myc gene and reduction of c-Myc protein level. Overall, this study showed an inverse correlation between HDAC7 and c-Myc expression in B-ALL patients (Barneda-Zahonero et al., 2015).

The role of c-Myc in CML

Chronic myeloid leukaemia (CML) is associated with fusion protein BCR-ABL1 and c-Myc that required for BCR-ABL mediated transformation (Xu, Voelter-Mahlknecht and Mahlknecht, 2005). However, the transformation defect of BCR-ABL SH2 deletion mutant and ABL C-terminal deletion mutant are deficient in activating c-Myc expression. Studies have shown that BCR-ABL induces c-Myc regulated transcription through E2F1 binding sites on c-Myc promoter site and increases c-Myc protein expression levels through activation of JAK2. Furthermore, it was also reported that activated JAK2 also protects c-Myc protein from 26S proteasome-dependent degradation (Xie et al., 2008). A study from Xu et al (2005) found that histone deacetylase inhibitor SAHA (suberoylanilide hydroxamic acid) decreased levels of BCR-ABL protein expression and c-Myc in BV-173 cells whereas only a minor decrease of BCR-ABL protein was observed in K562 cells. Thus, the study concluded that SAHA not only downregulates BCR-ABL expression but also inhibits expression of c-Myc in BV-173 cells and eventually inducing apoptosis. A recent study has identified a novel transcriptional repressor of c-Myc in CML, ZNF224 that is negatively regulated by BCR-ABL1. As a result of this, ZNF224 was able to inhibit cell proliferation and induces apoptosis in CML by downregulating c-Myc (Sodaro et al., 2018). In addition, another study in CML found that perturbations in both p53 and c-Myc led to synergistic kill, differentiation and near elimination of transplantable human leukemic stem cell (LSC) in mice (Abraham et al. 2016). Furthermore, another study found that there is an overexpression of c-Myc in the dmin-containing HL60 leukemic cell line but other sequences derived from 8q24 had even higher level of c-Myc expression (Sen et al., 1994).

The role of c-Myc in CLL

Chromosomal translocations involving c-Myc are rare in chronic lymphocytic leukaemia (CLL) (Huh et al., 2008). Expression of c-Myc is uniformly low or absent in CLL (Ferrari et al., 1985). Studies found that c-Myc oncogenes are expressed at significantly elevated levels or can be induced under certain *in vitro* conditions for neoplasia with low proliferative activities such as CLL (Greil et al., 1991). CLL with c-Myc rearrangement normally is associated with increased prolymphocytes, complex cytogenetic abnormalities and poor prognosis (Huh et al., 2008). A study investigating the function of CTLA4 expression on primary CLL cells, found that downregulation of CTLA4 caused upregulation of CD38/BCR signalling downstream molecules such as c-Fos, c-Myc, and BCL2, which resulted in prolonged survival and increased proliferation of CLL cells (Mittal, 2010). This is because CTLA4 expression is inversely correlated with CD38 expression in CLL cells which in an unfavourable prognosis (Malavasi et al., 2011; Mittal, 2010).

The role of c-Myc in MDS

RAEB-2 subgroup of myelodysplasia syndrome (MDS) and group with 11-20% blast cells was shown to have the highest expression of c-Myc among all MDS subgroups. Lowest c-Myc expression was found on patient with deletion of chromosome 5q and patient with RAEB-1. It was also shown that, patients with favourable karyotype have significantly lower expression of c-Myc than patients with intermediate and unfavourable karyotype (Poloni et al., 2013). In higher-risk myelodysplastic syndrome patients, there was overexpression of ribosomal protein (RP) L23 which was associated with abnormal apoptotic resistance in CD34+ cells. As RPL23 is encoded by a target gene of c-Myc, when there is increased expression of RP123 and c-Myc, Miz-1 expression is decreased and leads to depression of Miz-1-induced transcriptional activation of cell cycle inhibitors p15Ink4b and $p21^{Cip1}$. This RPL23/Miz-1/c-Myc regulation circuit provides a feedback loop that links efficient RPL23 expression with c-Myc's function to suppress Miz-1-induced CDK inhibitors and leads to apoptotic resistance. RPL23 suppression led to c-Myc downregulation and resulted in decreased cellular viability,

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increased apoptosis, and G1-S cell cycle arrest (Qi et al., 2017).

Clinical significance

In terms of clinical significance, it is also apparent that these genes have immense diagnostic and prognostic value. Therefore, it makes perfect sense to consider these genes as potential therapeutic targets. The first step in this direction would be to understand the regulations and interactions of these genes with other molecular pathways. Currently, the advent of next generation sequencing (NGS) which enables simultaneous interrogation of multiple genetic perturbations at the genome and transcriptome level has amassed a wealth of information regarding the mutational status of FOXO3 and c-Myc genes in leukaemia and MDS. Recent NGS data detected the presence of mutations in both c-Myc in AML and ALL; FOXO3 in AML and CLL, indicating that these mutations could play a part in disease development and progression (Leeksma et al., 2019; Liu et al., 2016; Kim et al., 2019; Kim et al., 2020).

Conclusion and Future Perspectives

This review has highlighted in depth that FOXO3 is a tumour suppressor and is important for leukemogenesis (Li et al., 2012). Similarly, c-Myc was shown to be essential for leukemogenesis (Salvatori et al., 2011). Nevertheless, the regulations and interactions of these genes with other molecular pathways have not been fully elucidated to date as shown in our review. Research into this area merits further investigation and holds much promise for as it would open doors for more molecular targeted therapy in leukaemia and cancer in general. The outcome would be especially beneficial for leukemic and cancer patients who are resistant to standard chemotherapy regimens.

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Conflict of Interests

The authors declare that there are no conflict of interests arising from the publication of this review article.

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