MYC in T-cell acute lymphoblastic leukemia: functional implications and targeted strategies

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Abstract

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological cancer that frequently occurs in children and adolescents, which results from the transformation of immature T-cell progenitors. Aberrant cell growth and proliferation of T-ALL lymphoblasts are sustained by activation of strong oncogenic drivers. Mounting evidence highlights the critical role of the NOTCH1-MYC highway toward the initiation and progression of T-ALL. MYC has been emphasized as a primary NOTCH1 transcriptional target impinging in leukemia-initiating cell activity particularly responsible for disease onset and relapse. These findings lay a foundation of T-ALL as an ideal disease model for studying MYC-mediated cancer. The biology of MYC deregulation in T-ALL supports innovative strategies for therapeutic targeting of MYC. To summarize the relevant literature and data in recent years, we here provide a comprehensive overview of the functional importance of MYC in T-ALL development, and the molecular mechanisms underlying MYC deregulation in T-ALL. Finally, we illustrate the innovative MYC-targeted approaches that have been evaluated in pre-clinical models and shown significant efficacy. Given the complexity of T-ALL molecular pathogenesis, we propose that a combination of anti-MYC strategies with conventional chemotherapies or other targeted/immunotherapies may provide the most durable response, especially for those patients with relapsed and refractory T-ALL.

Keywords: MYC, T-ALL, Targeted therapy

T-cell acute lymphoblastic leukemia (T-ALL) is one of the most common pediatric malignancies characterized by diffuse infiltration of bone marrow with malignant lymphoblasts expressing immature T-cell surface markers.[1] Although the prognosis of childhood T-ALL has improved markedly in recent years with an approximately 85% 5-year event-free survival, the remarkable success of pediatric treatment has not been achieved in adult patients.[2] The cure rate for T-ALL in adults is about 50% and overall survival at 5 years after relapse is only 7%.[3,4] Novel therapeutics are emergent for relapsed and refractory cases. Although chimeric antigen receptor T-cell therapy (CAR-T) has revolutionized the treatment of relapsed/refractory ALL, particularly in B-ALL with CD19-CAR-T,[5] CAR-T treatments in T-ALL, such as CD7, CD5, and CD1a CAR-T, are still in early clinical trials.[6–9]

Current research efforts are therefore still focused on identifying aberrant molecular pathways and targets for therapeutic intervention. In the past decade, genome-wide sequencing and transcriptomic studies have uncovered abundant oncogenes and tumor suppressors involved in T-ALL transformation. Among these genes, *MYC* is one of the most activated oncogenes in this hematological malignancy. Aberrant *MYC* expression drives T-ALL progression and maintains the activity of leukemia stem cells;[10] MYC inhibition effectively suppresses leukemogenesis. Notably, the essential role of MYC in T-ALL development has been solidly validated in multiple leukemia models.[11,12]

1. IMPORTANCE OF MYC IN T-ALL

The *MYC* family of oncogenes includes *MYC*, *MYCN*, and *MYCL* that encode the basic helix-loop-helix leucine zipper transcription regulators MYC, N-MYC, and L-MYC, respectively, and MYC is the most prevalent among the three.[13] The *MYC* oncogene is deregulated in >50% of human cancers. This deregulation is frequently associated with poor prognosis and unfavorable patient survival, and has been recognized as a major molecular event during cancer initiation and maintenance.[14] Enforced *MYC* expression in transgenic murine tissues of many varieties can trigger tumorigenesis, illustrating its transforming activity in vivo.[15–17] The MYC oncoprotein belongs to a family of so-called "super-transcription factor" that potentially regulates the transcription of at least 15% of the entire genome.[18] For a long time, MYC protein was considered to be a gene-specific transcription factor. This view has been challenged by the work demonstrating that MYC proteins bind to virtually all active promoters and many enhancers.[19,20] This controversy has been reconciled that specificity and generality maybe both involved in controlling downstream gene expression, and those genes containing consensus MYC binding sequence (E-box) should be more preferentially regulated by the MYC oncoprotein. Despite the discrepancy of mechanisms underlying transcriptional activation, it is undebatable that MYC contributes to almost every aspect of tumor cell biology, including vasculogenesis, metabolic reprogramming, genomic instability, and immune escape.[18]

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High levels of MYC expression are broadly present in T-ALL. MYC was originally implicated in the pathogenesis of T-ALL resulting from a rare t(8;14)(q24;q11) translocation, which induces MYC overexpression by placing the *MYC* gene under the control of strong T-cell-specific enhancer elements in the vicinity of the TCRA/TCRD.[21–23] Over the past decade, it becomes evident that *MYC* is primarily activated by the aberrant NOTCH1 signaling pathway. Gain-of-function mutations within the *NOTCH1* receptor are found in more than 65% of T-ALL patients, which intensify the signaling strength and result in aberrant *MYC* transcriptional activation.[24] Global chromatin immunoprecipitation mapping of NOTCH1 and MYC binding DNA sequences show that NOTCH1 and MYC control an overlapping repertoire of cell growth target genes,[25] underscoring the NOTCH1-MYC regulatory highway toward T-cell leukemogenesis. It is also interesting to note that some rare *MYCN* somatic mutations (~2.3%) were found in T-ALL, which was shown to prevent N-MYC protein degradation and induce T-lineage leukemia with significantly shorter latency in T-ALL models.[26] The important role of *MYCN* was further highlighted by the finding that *MYCN* is overexpressed in a large population (40–50%) of T-ALL patients, yet the functional significance and molecular mechanism remain to be investigated.[27]

Both genomic and functional analyses of MYC targets suggest that the MYC responsive gene network includes groups of genes involved in cell cycle regulation, metabolism, ribosome biogenesis, protein synthesis and mitochondrial function.[28] Many target genes of MYC involved in the cell cycle have been identified. Our research team has recently demonstrated MYC directly activates *AURKB*,[29] *WEE1*,[30] and *CHK1*[31] transcription in T-ALL. It is important to know that MYC not only induces the expression of cell cycle kinases that promote cell division such as AURKB but also checkpoint kinases (eg, WEE1 and CHK1) that prevent premature mitotic entry with unrepaired DNA damage. As a master oncogenic driver, MYC is required to balance between robust DNA replication and the ensuing DNA damage to maintain optimal proliferation and survival. As for the anti-apoptotic role, MYC was shown to transcriptionally suppress the expression of Bcl-2 interacting mediator of cell death (BIM), the pro-apoptotic factor. Of note, BIM repression is a key cell survival signal in the molecular pathogenesis of high-risk T-ALL and its derepression effectively induces apoptosis in primary treatment-resistant T-ALL.[32] In addition, MYC regulates cancer cell metabolism through its direct activation of genes involved in metabolic pathways such as glycolysis.[18] Several key enzymes of glucose metabolism are well-characterized MYC targets, which was also validated in the T-ALL context. We found that the WEE1-MYC axis in T-ALL enhances glucose uptake and glycolysis. As such, pharmacological inhibition of WEE1 elicits MYC downregulation, which results in marked suppression of aerobic glycolysis and robust cell death.[30] Taken together, these cell cycle, anti-apoptotic and metabolic targets exemplify that the MYC target gene network plays a major role in driving leukemogenic program.

MYC serves an essential role in both T-cell development and transformation. Constitutive and cell-autonomous signals emanating from the pre-T-cell receptor (pre-TCR) promote proliferation, survival and differentiation of immature thymocytes to the $CD4^+CD8^+$ double-positive (DP) stage. During this process, MYC is rapidly upregulated upon induction of the pre-TCR signaling and diminished by the DP stage. Loss of *MYC* in the thymus causes markedly reduced thymocyte numbers as a result of suppressed cell proliferation and growth. Persistent activation of MYC signaling throughout the DP stage induces neoplastic transformation.[33] Strong oncogenic activity of MYC in T-ALL has been solidly demonstrated in mouse and zebrafish leukemia models. Sustained expression of the *MYC* transgene in hematopoietic cells culminates in the formation of malignant T-cell lymphomas in mice.[17] Expression of *MYC* under the control of lymphatic-specific rag2 promoter has shown to induce T-ALL in zebrafish.[11] Moreover, multiple lines of evidence suggest that MYC plays an indispensable role in leukemia-initiating cell maintenance and self-renewal in T-ALL, and MYC protein abundance is considered as a *bona fide* LIC marker in human T-ALL. Functional studies from T-ALL mouse models demonstrate *MYC*-expressing leukemic cells are more readily to reconstitute leukemogenesis in the secondary transplants.[10] More importantly, suppression of *MYC* by small hairpin RNA or the bromodomain and extra-terminal (BET) bromodomain BRD4 inhibitor JQ1[34] significantly prevents leukemia initiation and decreases leukemia burden in mice by specifically targeting the LIC fraction.[10,12] In a nutshell, MYC involvement in both T-ALL initiation and maintenance suggests that therapeutic efforts aimed at inhibiting MYC expression or activity should have an important clinical relevance. Since targeting of MYC is limited by direct strategy, understanding the regulatory mechanism underlying MYC deregulation becomes a key route for indirect targeting approaches.

2. MYC REGULATORY NETWORK IN T-ALL

2.1. Transcriptional regulation

Molecular mechanisms governing *MYC* expression in T-ALL are complex and diverse. Of particular note, *MYC* is a major downstream target gene of the NOTCH1 signaling pathway.[35,36] Identification of direct binding of NOTCH1 at a distal enhancer near the *MYC* locus (NOTCH1-controlled *MYC* enhancer, N-Me) provides the most straightforward evidence of NOTCH1 activating *MYC* transcription. N-Me is a highly conserved regulatory element located 1.27 megabases (Mb) 3′ from the transcriptional start site (TSS) of the mouse *Myc* gene and 1.4 Mb from the TSS of the human *MYC* gene.[37,38] Interestingly, recurrent somatically acquired focal duplications of the N-Me were found in 5% of T-ALL cases, consolidating the oncogenic *MYC* enhancer activity in T-ALL.[37] Moreover, GATA3-driven nucleosome eviction dynamically modulates N-Me enhancer activity and is strictly required for NOTCH1-induced T-ALL initiation and maintenance. Mechanistically, GATA3 binding facilitates the recruitment of SWI/SNF chromatin-remodeling complexes mediating an open chromatin configuration to N-Me, enabling stimulating-transcription factor control of *MYC* expression.[39] In addition to GATA3, RUNX1 (Runt-related transcription factor 1) is also required for intracellular NOTCH1 binding and chromatin accessibility at the N-Me. RUNX1 depletion results in increased histone 3 loading and elicits a closed chromatin configuration to reduce chromatin accessibility at the N-Me.[40] Transcriptional activation of *MYC* involves a core-transcriptional complex consisting of intracellular NOTCH1, CSL, and MAML. It is also shown that, in a T-ALL mouse model, a previously unrecognized co-activator ZMIZ1 directly interacts with NOTCH1 and selectively activates *MYC* expression.[41]

While the expression of *MYC* oncogene is regulated by NOTCH1 through binding to N-Me, this link between NOTCH1 and MYC is bidirectional mediated by microRNA-30a (miR-30a). miR-30a is able to bind to the 3′ untranslational region (3′ UTRs) of *NOTCH1* and *NOTCH2*, inhibiting their expression. In T-ALL, miR-30a remains very low due to MYC-mediated

transcriptional repression. As such, intracellular NOTCH1 and NOTCH2 induce expression of *MYC*, which in turn suppresses miR-30a, thereby constitutes a miR-30a-NOTCH-MYC feedforward loop amplifying the NOTCH-MYC leukemogenic program in T-ALL.[42]

2.2. Post-transcriptional regulation

Post-transcriptional regulation involves RNA splicing and RNA stability, which plays an indispensable role in MYC expression as well. We previously demonstrated that SHQ1, an H/ACA snoRNP assembly factor involved in snRNA pseudouridylation on spliceosomal RNA, is highly expressed in T-ALL. RNA-Seq reveals that *SHQ1* depletion impairs widespread RNA splicing, and *MYC* is one of the most prominently downregulated genes due to inefficient splicing. Specific regulation of *MYC* splicing by SHQ1 mostly relies on the unique intronic branch site sequence of *MYC* that is preferentially base paired with pseudouridine (Ψ) in U2 snRNA. Importantly, *MYC* overexpression significantly rescues T-ALL cell death resulted from *SHQ1* inactivation. These findings add an extra layer of complexity to *MYC* regulation at the RNA splicing level.[43]

In addition, RNA degradation machinery is also implicated in the regulation of MYC by modulating RNA stability of E3 ligase and deubiquitinase that regulate MYC protein turnover. Nudix hydrolase 16 (*NUDT16*), shown to have mRNA decapping activity, is transcriptional silenced by its promoter CpG island hypermethylation in T-ALL. This epigenetic defect of *NUDT16* prevents decapping and subsequent decay of target RNAs involved in cellular transformation. Specifically, NUDT16 destabilizes mRNA targets such as the *FBXO28* and *USP37* transcripts. Instead of eliciting protein degradation, FBXO28 ubiquitin ligase activity promotes MYC-driven transcription by stimulating MYC-p300 interactions at the target promoters.[44] USP37 has been shown to stabilize MYC in lung cancer.[45] As a result, disrupted RNA decapping pathway by *NUDT16* silencing promotes *FBXO28* and *USP37* expression, and leads to stabilized MYC with amplified transcriptional activity.[46]

2.3. Post-translational regulation

Post-translational regulation of MYC protein, including various modifications associated with altered MYC protein stability or activity, has been rigorously studied in the past decade and considered as the most exquisite mode of regulation in various cellular contexts. The well-defined event in MYC degradation involves sequential phosphorylations of two critical residues within MYC Box I domain, serine 62 (S62), and threonine 58 (T58).[47] Work in several laboratories has elucidated a signaling pathway that regulates these phosphorylation events. Following cell growth stimulation, MYC is phosphorylated at the serine 62 (pS62) by ERK and/or CDKs, which primes subsequent phosphorylation at threonine 58 (pT58) by GSK-3β.[48] S62 phosphorylation can be removed by protein phosphatase 2A (PP2A),[49] MYC pT58 is then subjected to polyubiquitination by FBXW7 and protein degradation by the 26S proteasome.[50]

Highlighting the importance of this degradation pathway in cancer, many of the signaling proteins implicated in the MYC S62/T58 phosphorylation are often deregulated in tumor cells, resulting in altered MYC phosphorylation and increased MYC protein stability. In T-ALL, MYC can be enormously highly expressed in a large number of cases absent of *NOTCH1* gain-of-function mutations, highlighting the role of non-transcriptional regulation of MYC expression. Indeed, regulations at the protein stability level are involved. Aberrant activation of PI3K/AKT due to *PTEN* loss-of-function mutations phosphorylates and inactivates GSK3β, preventing the subsequent phosphorylation at MYC T58.[51] It is therefore believed that inactivation of tumor suppressor PTEN contributes to MYC accumulation by enhancing PI3K/AKT. These findings constitute an alternative pathway for MYC activation in T-ALL, and lend further support to the significance of targeting the PTEN/AKT pathways in T-ALL treatments.

FBXW7 encoding the primary E3 ligase responsible for the MYC protein degradation is mutated in a significant portion of human tumors,[52] including approximately 20% of patients with T-ALL.[53,54] Mutations in *FBXW7* are predominantly heterozygous and clustered within the WD40 substrate-binding domain and involve amino acid changes, resulting in reduced capacity of polyubiquitination.[54] To investigate the transforming effects of such missense mutations, inducible knockin models of *FBXW7* mutations were generated, mimicking the most common substitution found in human T-ALL (R465C). Interestingly, the substitution of arginine to cysteine does not compromise normal hematopoietic stem cell (HSC) function but leads to a marked increase in the proportion of leukemia-initiating cells. *FBXW7* mutations were shown to specifically affect the ubiquitylation and half-life of MYC protein. Based on an exquisite GFP-MYC knockin mouse model, the function of FBXW7 was strongly linked to MYC abundance and MYC expression was significantly correlated to leukemia-initiating activity.[10] These findings not only shed light on the crucial role of MYC in T-ALL LIC enrichment but also provide strong evidence demonstrating the key regulatory role of FBXW7 in MYC stabilization.

Our research team has recently identified an unconventional mechanism involving MYC modification and proteasomal degradation. We revealed that Aurora B kinase (AURKB) is a *bona fide* MYC binding partner, which directly phosphorylates MYC at the serine 67 (S67). This MYC phosphorylation is distinct from the S62 phosphorylation in the canonical MYC degradation pathway. The S67 phosphorylation blocks the interaction between MYC and GSK3β, thereby inhibiting the T58 phosphorylation and subsequent FBXW7-mediated proteasomal degradation. We constructed MYC non-phosphorylatable alanine mutants (S67A) and phosphomimetic aspartic acid mutants (S67D) respectively, and performed a series of cycloheximide chase experiments demonstrating the S67 phosphorylation stabilizes MYC protein. Interestingly, stabilized MYC, in concert with T-cell acute lymphoblastic leukemia 1 (TAL1), directly activates *AURKB* transcription, constituting a positive feedforward loop that reinforces MYC-regulated oncogenic programs. Using a state-of-the-art T-ALL zebrafish model, we provided strong evidence supporting that AURKB-mediated MYC phosphorylation accelerates leukemogenesis. We therefore conclude that the S67 phosphorylation of MYC is associated with the tumor-promoting effect of AURKB and is functionally important for MYC stabilization and T-cell leukemogenesis in vivo. As such, inhibitors of AURKB induce prominent MYC degradation concomitant with robust leukemia cell death.[29] These findings reveal a unique MYC modification impinging in T-cell leukemogenesis.

Deregulation of the pathway that modulates MYC post-translational modification represents an important mechanism for oncogenic activation of MYC in human cancers. Although MYC can be modified at different levels by multiple pathways, adding more complexity to understand the regulatory network (Fig. 1), this intricacy may also become therapeutic vulnerability and a focus for therapeutic targeting.

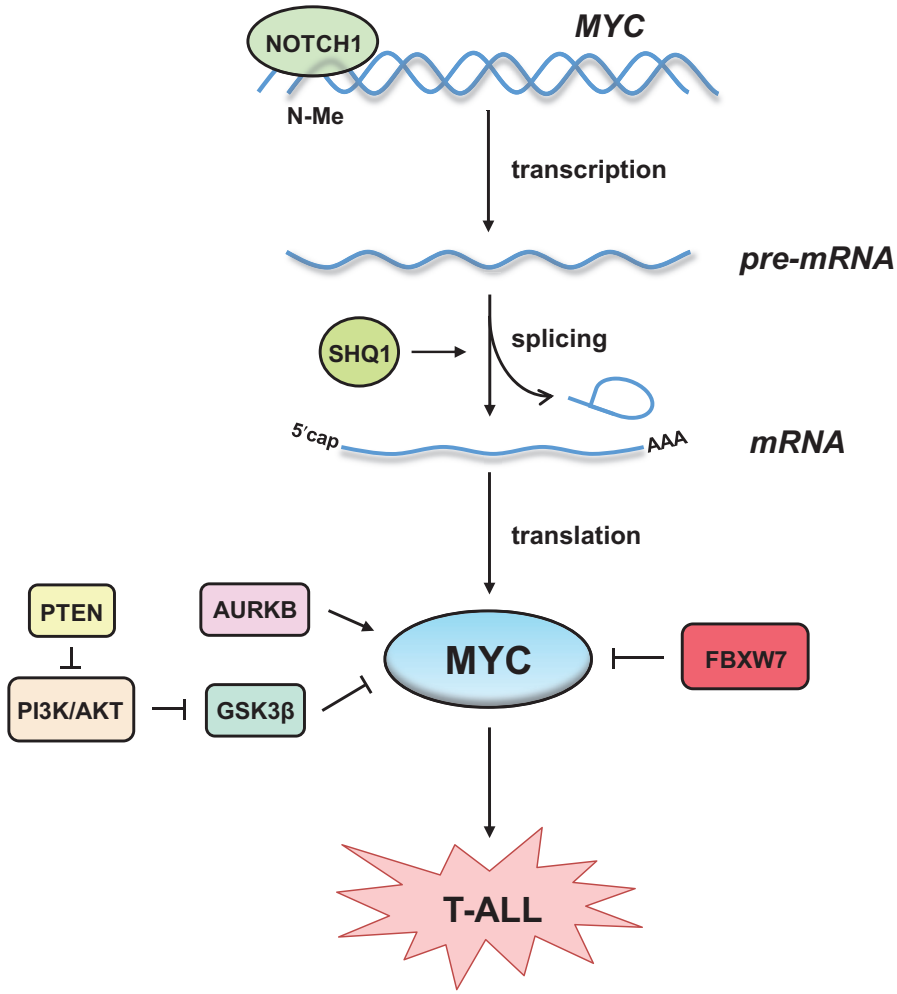

Figure 1. Schematic presentation of the major MYC regulatory network in T-ALL. MYC can be regulated at multiple layers. At the transcriptional level, *MYC* is a major target gene downstream of NOTCH1. Activating NOTCH1 mutations produces more abundant intracellular NOTCH1, which directly binds to N-Me, a distal enhancer of *MYC*, and activates *MYC* transcription. At the post-transcriptional level, SHQ1 promotes *MYC* RNA splicing. At the post-translational level, MYC is subjected to GSK3β-mediated phosphorylation, then undergoes polyubiquitination by FBXW7 and proteasomal degradation. Recurrent *FBXW7* inactivating mutations in T-ALL leads to MYC accumulation. AURKB directly phosphorylates MYC and counteracts GSK3β-directed phosphorylation and subsequent proteasomal degradation. Frequent PTEN loss in T-ALL activates PI3K/AKT, which in turn phosphorylates and disables GSK3β, resulting in MYC stabilization.

3. THERAPEUTIC TARGETING OF MYC IN T-ALL

Although the prognosis of T-ALL has steadily improved with the help of modern chemotherapy, intensive T-ALL chemotherapy still faces the challenges of significant side effects, particularly to young patients. Targeted strategies with greater efficacy and lower toxicities are in desperate need. The important role of MYC in T-ALL makes it particularly important to design MYC modulators as a targeted approach for T-ALL treatment. However direct targeting of MYC has remained unsuccessful owning to its "undruggable" protein structure, and indirect inhibition of MYC has emerged as an alternative strategy.

Indirect targeting of MYC has been vigorously investigated for decades. These strategies include inhibition of *MYC* gene transcription or MYC-dependent transcriptional signaling. Accumulating evidence demonstrates that MYC-overexpressing tumor cells are subjected to transcriptional addiction.[55] Small molecule JQ1 selectively binds to the acetyl-lysine recognition motif of BET family member BRD4, resulting in repression of *MYC* transcription.[34] The covalent CDK7 inhibitor THZ1 and inhibitors of the super elongation complex (SEC) KL1/2 are shown to inhibit MYC transcriptional network and impede MYC-mediated cancer progression.[56–58] Alternatively, a variety of protein modifications is dynamically involved in regulating MYC expression. Targeting these upstream signals involved in the post-translational regulation of MYC has been identified as an important surrogate to reduce MYC activity. For instance, MYC protein is deubiquitinated and stabilized by the ubiquitin-specific protease (USP) family members USP7,[59] USP28,[60] and USP36.[61] USP7 inhibitor P22077 markedly decreases N-MYC protein and suppresses tumorigenesis.[59] Moreover, long-term efforts have focused on preventing MYC interaction with MAX, the "partner in crime," which is essential for MYC-mediated transcription program. The peptide mimetic IIA6B17 was first reported as a small-molecule inhibitor of the MYC-MAX dimerization.[62] Small molecule compounds MYCi361 and MYCi975 are the most recently reported inhibitors that disrupt the MYC-MAX complex, enhance MYC degradation and exhibit potent anti-tumor efficacy.[63] Finally, given MYC overexpression is frequently detected in human cancers, blocking genes exhibiting synthetic lethality with overexpression of MYC may show a killing effect only in cancer cells but spare normal counterparts. Inhibitors that target cyclin-dependent kinase 1

(CDK1) and DNA-damage checkpoint kinase (CHK1) selectively induce apoptosis in MYC-overexpressing tumor cells.[64,65] Our group has also shown that proteasome inhibitor Bortezomib elicits a synthetic lethal effect on *MYCN*-amplified neuroblastoma.[66]

As for anti-T-ALL efficacy, small molecular inhibitors that block *MYC* transcription are well-studied. Inhibition of BRD4 with JQ1 significantly induces profound downregulation of *MYC*, resulting in potent apoptosis in murine and human T-ALL cells.[12] Moreover, an shRNA knockdown screen to identify chromatin regulators essential for viability of anti-NOTCH-resistant T-ALL cells revealed BRD4. BRD4 binds enhancers near-critical T-ALL genes, including *MYC* and *BCL2*. The BRD4 inhibitor JQ1 downregulates the expression of these targets and induces growth arrest and apoptosis in resistant cells. JQ1 in combination with anti-NOTCH treatment was shown effective against primary human leukemias *in vivo*.[67] As further support of this notion, JQ1 suppresses leukemia initiation by eliminating leukemia-initiating cell activity in T-ALL,[10] and treatment with JQ1 significantly inhibits the growth of relapsed and induction failure T-ALL cells.[12] These findings establish an important role for BRD4 and MYC in leukemia resistance that may be addressed by treatment of selective BRD4 inhibitors or in combination with other drugs. Although BRD4 as a novel drug target has yielded many anti-cancer drugs that are now in clinical trials, initial evaluation of treatment with some BET-inhibitors revealed toxic side effects and their therapeutic significance still needs to be validated.[68]

We previously reported the AURKB-MYC regulatory circuit contributing to T-cell leukemia, which presents a novel therapeutic approach for targeting MYC by AZD1152, a selective AURKB inhibitor currently being evaluated in clinical trials. AURKB directly phosphorylates MYC at serine 67 and contributes to MYC stability. Inhibitors of AURKB induce prominent MYC degradation and attenuate the MYC transcriptional activity concomitant with robust leukemia cell death. Notably, inactivating *FBXW7* mutations predict resistance to AZD1152-based therapies due to the incapability of these T-ALL cells to elicit efficient MYC degradation. Moreover, an FDA-approved drug screen manifests that combined treatment of AZD1152 and vincristine synergistically suppresses tumor progression in T-ALL patient-derived xenografts.[29] This study not only sheds light on an unconventional mode of MYC phosphorylation-mediated protein stabilization but also provides a mechanism-based therapeutic strategy to target MYC and treat T-ALL. These data propel an immediate clinical trial of AURKB inhibitors in the treatment of T-ALL, or perhaps other MYC-dependent cancers with *FBXW7* mutational status as a biomarker.

4. CONCLUSION

The central role of MYC in tumorigenesis has been solidly documented in T-ALL as well as many other cancer types. In particular, MYC-induced T-ALL mouse and zebrafish models have successfully recapitulated human disease. These findings render T-ALL as an excellent pathogenic context for studying MYC-mediated cancer and evaluating the efficacy of anti-MYC approaches. With the hurdles of developing potent, direct-acting MYC inhibitors, deciphering unique mechanisms underlying MYC deregulation in T-ALL and developing indirect targeting strategies may be more feasible. Combination of anti-MYC strategies with conventional chemotherapies, targeted therapies, or immunotherapies should be encouraged not only for an in-depth understanding of pathogenic mechanisms but also for potential clinical translation to benefit T-ALL patients, particularly with relapsed disease or induction failure.

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