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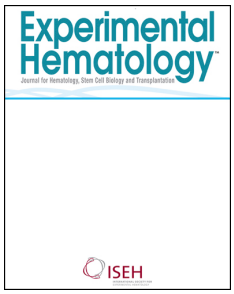

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Targeting the transcription factor Myb by small molecule inhibitors

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Highlights

The transcription factor Myb is an emerging drug target for leukemia therapy.

Initial work has identified several Myb-inhibitory compounds providing proof-of-principle that targeting of Myb is feasible.

Small-molecule suppression of Myb activity preferentially inhibits proliferation of leukemic versus normal hematopoietic cells.

Abstract

The transcription factor Myb is a key regulator of hematopoietic cell proliferation, differentiation and survival and has been implicated in the development of leukemia and several other human cancers. Pharmacological inhibition of Myb is therefore emerging as a potential therapeutic strategy. Recently, the first low molecular weight compounds that show Myb inhibitory activity have been identified. Characterization of these compounds suggests disruption of the protein-protein-interaction of Myb and the co-activator p300 as a suitable strategy to inhibit Myb.

The transcription factor Myb

Myb was initially discovered as the protein encoded by the cellular counterpart (c-*myb*) of the retroviral oncogene (v-*myb*) of the avian myeloblastosis virus. Together with two related proteins, A-Myb (or Mybl1) and B-Myb (or Mybl2), Myb forms a family of transcription factors in humans and other vertebrates, whose members share a highly conserved DNA-binding domain but have distinct functions and expression patterns. Myb is highly expressed in immature cells of all hematopoietic lineages and plays a key role as a regulator of hematopoietic cell proliferation, differentiation and survival [1,2]. Myb-null mice die during embryonic development and show multiple defects of almost all hematopoietic lineages, demonstrating that Myb is essential for the development of the hematopoietic system [3]. Studies with conditional Myb knock-out mice have identified distinct steps during the differentiation of the different lineages that are dependent on Myb expression [4,5,6,7]. Myb

is also expressed, although at lower levels, in certain epithelial cell types, such as the stem cells of the intestinal epithelium [8]. By contrast, A-Myb is mainly expressed in the male germ line, in the female breast epithelium and in B-cells whereas B-Myb is ubiquitously expressed in proliferating cells and functions as a cell cycle-dependent transcription factor [2,9].

The Myb protein structure consists of an N-terminal DNA binding domain that recognizes the consensus nucleotide motif PyAAC$^{T}/_{G}$G, a transactivation domain located in the center of the protein and a C-terminal negative-regulatory domain. A variety of proteins interacting with Myb have been identified and implicated in regulating its activity (Fig.1). Of these proteins the co-activator p300 plays a pivotal role as a Myb interaction partner. p300 is recruited to Myb via its so-called KIX domain that binds to a LXXLL amino acid motif located in the transactivation domain of Myb [10]. Importantly, mouse strains that are defective in the Myb-KIX interaction due to mutations of the LXXLL-motif or the KIX domain show multiple hematopoietic defects [11,12,13], pinpointing an essential role of p300 as a cooperation partner of Myb in the hematopoietic system. Structural analyses of a complex of the KIX-domain and a short peptide containing the LXXLL-motif of Myb have revealed that this motif and the adjoining amino acids form an α-helix that binds to a hydrophobic groove on the surface of the KIX domain [10].

Role of Myb in human leukemia

The initial discovery of v-*myb* as the oncogene responsible for the ability of AMV to cause myeloid leukemia in chickens suggested that Myb might also play a role in human leukemia. Although several studies showed that Myb is highly expressed in most human acute myeloid and lymphoid leukemias [14], it remained open whether high Myb expression plays a causal role in the development of the leukemia or is just a consequence of the immature phenotype of the leukemic cells. However, with the emergence of sophisticated and sensitive methods to detect chromosomal alterations, recurrent translocations and duplications of the *MYB* locus were described in T-cell acute lymphoid leukemias [15,16,17], providing clear evidence for a role of *MYB* in the etiology of this type of leukemia. Subsequent work reported genomic

rearrangements of Myb in acute myelomonocytic and basophilic leukemia [18,19,20]. Furthermore, mutations generating *de novo* Myb binding sites upstream of the *TAL1* oncogene were recently detected in a significant fraction of T-ALL of children [21]. These changes were shown to create „super-enhancers“ that lead to over-expression of Tal1 which, in turn, causes the leukemia. Together, these findings have provided strong evidence that Myb plays a causal role in the development of certain leukemias. However, it has now been recognized that Myb plays also an essential role as a „maintenance factor“ in leukemias caused by genetic lesions of other genes, such as the recurrent chromosomal translocations that lead to the expression of various fusion proteins, including AML-ETO, CBFb-SMMHC, PML-RARa, MLL-AF9, MLL-ENL and others. Acute myeloid leukemias caused by these translocations show high levels of Myb expression that are essential for the maintenance of the leukemic cells. This has initially been observed in studies with Myb antisense oligonucleotides [22,23] and confirmed by more recent studies [24,25,26,27]. The concept that has emerged from this work is that the survival and proliferation of the leukemic cells requires high levels of Myb acting downstream of the oncogenic driver mutations. Importantly, the leukemic cells require - or are „addicted“ to - higher levels of Myb expression than normal hematopoietic progenitor cells. This phenomenon provides a rationale for a therapeutic approach based of Myb inhibition because it predicts that leukemic cells will be more sensitive to inhibition of Myb than normal hematopoietic progenitor cells. This idea is supported by recent studies of a mouse model of acute myeloid leukemia (AML), which showed that shRNA-mediated down-regulation of Myb resulted in remission of the leukemia without inhibiting normal hematopoiesis [26].

Myb has also been implicated in the development of non-hematopoietic tumors, such as breast and colon cancer [1], adenoid cystic carcinoma, a rare and currently intractable tumor which harbors translocations of the *MYB* and *NFIB* genes in a large proportion of cases [28], and diffuse low-grade pediatric gliomas [29]. Myb is therefore now regarded as a relevant target whose inhibition might open up novel strategies for the treatment of leukemia and other types of cancer.

Targeting Myb by small molecule inhibitors

The idea that Myb may be a relevant therapeutic target has prompted us to search for low molecular weight compounds as potential Myb inhibitors. We have established a cell-based inhibitor screening system that employs a myelomonocytic chicken cell line carrying an inducible Myb expression system and a GFP-based reporter gene, which is driven by the promoter and enhancer of the highly Myb-inducible chicken *mim-1* gene. Hence, the fluorescence of these cells is a direct measure of Myb activity. We initially used the cells to screen a collection of so-called sesquiterpene lactones (STLs) for Myb-inhibitory activity [30,31]. STLs are a large class of plant-derived natural compounds that have various biological activities. Surprisingly, already the first compound tested, Mexicanin-I, inhibited Myb activity in our reporter system at a low micromolar concentration. Further analysis showed that Mexicanin-I was able to inhibit the expression of *bona fide* Myb target genes and to suppress the proliferation of leukemia cell lines and of primary leukemic cells from several AML patients [30]. Although this study did not reveal how Mexicanin-I exerts its inhibitory effect, it showed for the first time that Myb activity can be inhibited by a low molecular weight compound. This finding was corroborated by the observation that Naphthol AS-E phosphate, a compound that was previously shown to bind to the KIX domain of p300 and to inhibit its interaction with the transcription factor CREB [32], also inhibits Myb activity [33]. Consistent with its effect on the KIX-CREB interaction it was shown that Naphthol AS-E phosphate disrupts the interaction between Myb and the KIX domain. This demonstrated that disruption of the Myb/KIX protein-protein-interaction with a drug-like small molecule is feasible. Subsequently, we have identified additional compounds that target the cooperation of Myb and p300. We showed that Celastrol, a triterpenoid isolated from roots of *Tripterygium wilfordii* (Thunder God Vine), potently inhibits Myb activity (EC50 approx. 1 μM) by blocking the Myb-p300 interaction [34]. Molecular docking studies suggest that Celastrol is able to bind to the hydrophobic groove on the surface of the KIX domain that is occupied by the α-helical LXXLL-motif in the Myb-KIX complex, suggesting that Celastrol competes

with Myb for binding to the KIX domain. Further work showed that Celastrol down-regulates the expression of authentic Myb target genes in HL60 cells and induces differentiation and apoptosis, as expected if Myb activity is inhibited. Importantly, expression of an activated version of Myb in HL60 cells rescued the effects of Celastrol on differentiation and apoptosis, indicating that these effects were indeed mediated by inhibition of Myb. Celastrol also inhibited the proliferation of primary AML cells derived form a mouse model of AML and from AML patients. Importantly, the proliferation normal murine and human early hematopoietic progenitor cells was not affected under the same conditions, consistent with the notion that AML cells require higher levels of Myb activity than their normal counterparts and, hence, are more vulnerable to inhibition of Myb than the normal cells. Finally, it was shown that Celastrol significantly prolonged the survival of mice in an *in vivo* model of an aggressive AML [34]. Overall, this work suggested that small molecule inhibition of Myb might have therapeutic potential for the treatment of AML.

Compounds binding to p300 are likely to have additional inhibitory activities because p300 is a co-activator of many transcription factors. We were therefore particularly interested in identifying compounds that act directly on Myb rather than on p300. Recently, we have shown that 5-hydroxy-2-methyl-1,4-naphthoquinone (also known as Plumbagin) and several related naphthoquinones bind directly to the transactivation domain of Myb and inhibit Myb activity [35]. Like Celastrol, Plumbagin induced the differentiation of HL60 cells and inhibited the proliferation of primary AML cells while the proliferation of normal progenitor cells was not affected. Naphthoquinones are well known for their ability to induce reactive oxygen species, however, this appeared not to be involved in the inhibition of Myb activity. Rather, Plumbagin appeared to disrupt the cooperation of Myb and p300. The 3-dimensional structure of the Myb transactivation domain is currently not known, nevertheless these findings suggest that this part of Myb displays a “binding pocket” that is amenable to binding by small molecules. Although Plumbagin is already quite active (EC50 approx. 0.5 μM) its rather simple chemical structure suggests that chemical approaches could be used to develop it further to more active compounds or to compounds that will minimize off-target effects. It will also be very

interesting to screen for further compounds that bind to the Myb transactivation domain, to eventually identify lead structures that can be developed into specific Myb inhibitors. Furthermore, in vivo leukemia models will be important tools to validate the targets being affected by the candidate drugs and to assess their therapeutic efficacy.

Summary

Myb is now regarded as a promising therapeutic target whose inhibition might open-up new strategies for the treatment of leukemia and of other tumors driven by deregulated Myb. Although the development of specific inhibitors for Myb is still in its infancy, initial attempts to find inhibitory compounds have successfully demonstrated that small molecule inhibition of Myb is feasible and may have potential for the therapy of AML. These studies have pinpointed disruption of the Myb/p300 protein-protein-interaction as suitable strategy to inhibit Myb activity. Thus, the stage is now ready for more sophisticated and extensive approaches to search for highly active small-molecule Myb inhibitors.

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Conflict of interest disclosure

The authors declare no conflict on interest.

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Figure legends

Figure 1. **Myb interaction partners**. The functional domains and several known interaction partners of Myb are shown schematically. LXXLL refers to an amino acid motif in the transactivation domain that mediates the interaction with p300.

Figure 2. **Proposed inhibitory mechanisms of Celastrol and Plumbagin**. The formation of a complex through the LXXLL motif of Myb and the KIX domain of Myb is shown schematically. Celastrol and Plumbagin are shown interfering with the Myb/p300 interaction by primarily targeting the KIX domain or the Myb transactivation domain.

Figure1

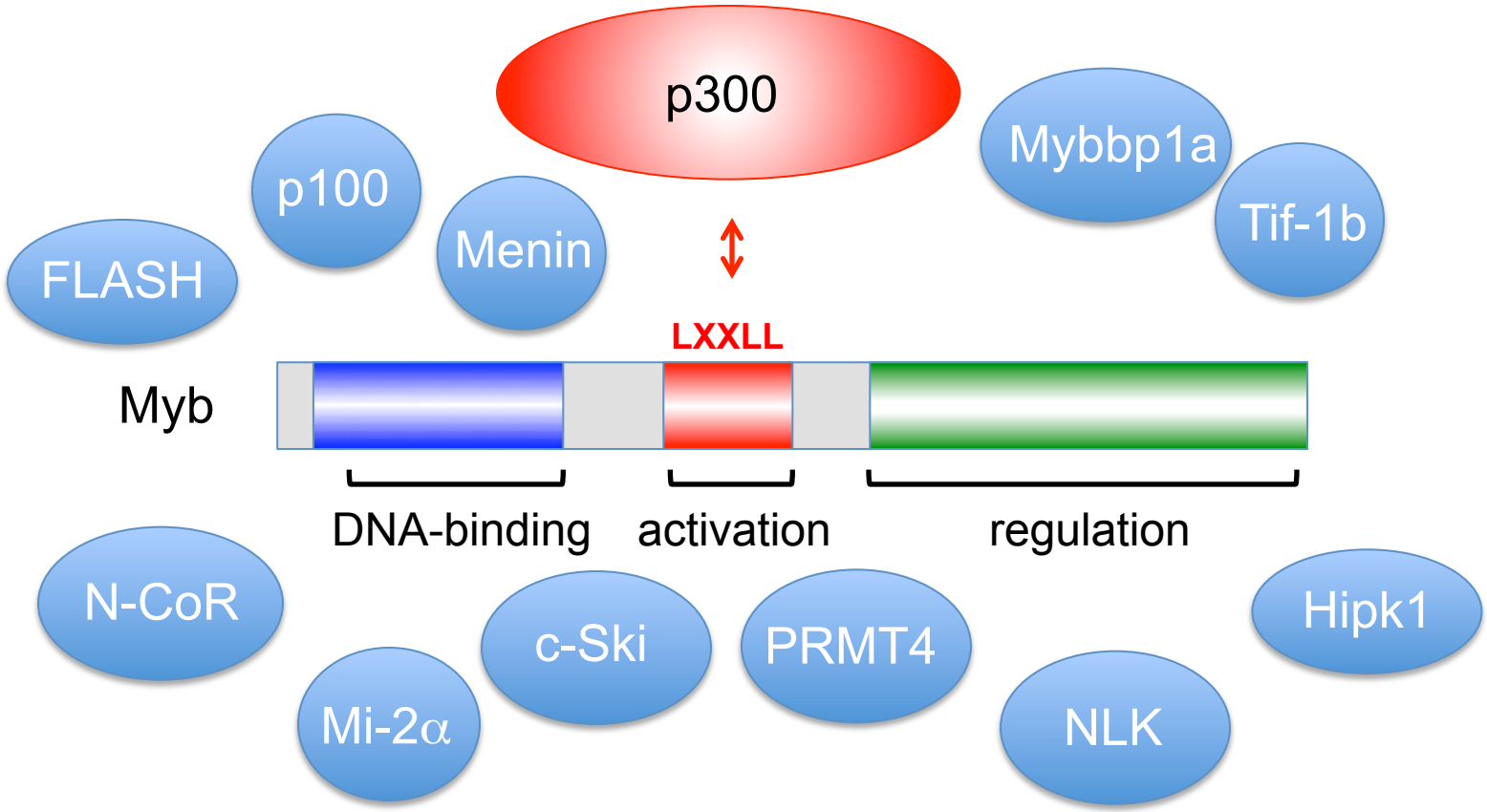
p300
p100
Menin
Mybbp1a
Tif-1b
FLASH
LXXLL
Myb
DNA-binding
activation
regulation
N-CoR
c-Ski
PRMT4
Hipk1
Mi-2α
NLK

Figure 2