ELSEVIER

Blood 142 (2023) 3638–3639

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Rapid-Kinetics Degron Benchmarking Reveals Off-Target Activities and Mixed Agonism-Antagonism of MYB Inhibitors

Taku Harada[1], *Monika Perez*[1], *Jeremie Kalfon*[2], *Rashad Batley*[1], *Flora Dievenich Braes*[1], *Stuart H Orkin, MD*[3,4,5], *Maxim Pimkin, MD PhD*[6]

[1] Boston Children's Hospital, Boston, MA
[2] Broad Institute, Cambridge, MA
[3] Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, MA
[4] Harvard Medical School, Boston, MA
[5] Howard Hughes Medical Institute, Boston
[6] Dana-Farber Cancer Institute, Brookline, MA

The transcription factor (TF) MYB is a critical transcriptional dependency of acute myeloid leukemia (AML) where it has been a long-term focus of drug development efforts. However, development of transcriptional inhibitors is hampered by the lack of a generally accepted functional cellular readout to characterize their target specificity and on-target activity. We reasoned that the specificity and on-target activity of MYB inhibitors would be best evaluated in a rapid kinetics system where their immediate transcriptional effects are benchmarked against targeted MYB degradation. We began by engineering a chemical degron model and established the direct gene-regulatory functions of MYB in a nascent transcriptomics assay. We fused the C-terminus of MYB with an FKBP12 ^{F36V} (dTAG) domain and a fluorescent tag by a homozygous knock-in of the FKBP12 ^{F36V}-coding DNA sequence into the endogenous MYB locus in MV411 cells. The resulting fusion protein was nearly completely degraded after a 1-hour treatment with dTAG ^{V}-1, a highly specific VHL-engaging PROTAC. As expected, degradation of MYB resulted in a profound loss of cell viability, consistent with the effects of a genetic MYB knockout in AML cells. To establish MYB's direct gene-regulatory functions, we measured genome-wide rates of nascent mRNA synthesis by thiol (SH)-linked alkylation metabolic sequencing of RNA (SLAM-seq) after a 1-hour MYB degradation. Defining direct targets as those genes which displayed significant changes in transcription rates (FDR<0.0), we detected 450 genes directly regulated by MYB. Of these, 319 genes were downregulated and 131 genes were upregulated, indicating that MYB acts as a transcriptional activator and repressor of these genes, respectively. In parallel, we performed SLAM-seq in AML cells treated with six agents reported as MYB inhibitors: MYBMIM, celastrol, naphthol AS-E phosphate, mebendazole, plumbagin and all-trans retinoic acid (ATRA). For comparison, we treated MV411 cells with the BET bromodomain inhibitor JQ1, which has been reported to indirectly inhibit MYB by interfering with its expression and function. The MYB inhibitors displayed a dramatic variability in the number of dysregulated genes, varying from 19 (naphthol) to 1123 (MYBMIM). In contrast, JQ1 caused widespread and bimodal effects, altering the transcription rates of >2000 genes in both directions. On pairwise overlap, the MYB inhibitors captured a relatively minor portion of the direct MYB program (between 5-155, or 1-34%, of the 450 direct MYB targets), compared with 43% of the MYB program captured by JQ1. Nonetheless, the MYB inhibitors displayed a stronger specificity for MYB target genes compared to JQ1, because they elicited much narrower responses. Thus, while MYB inhibitors displayed strong enrichments for primary MYB targets, they did not attenuate the entire MYB transcriptional program, and significant portions of their activities appeared to be off-target. In addition, the inhibitors displayed bimodal effects on the transcription of MYB-regulated genes, further activating subsets of genes that were repressed by MYB degradation, and vice versa. These observations uncovered unexpected activities of MYB inhibitors as context-dependent mixed agonists-antagonists. We conclude that MYB has a narrow direct transcriptional program in AML cells, which is only partially captured by the existing MYB inhibitors, which display partial specificity for MYB targets and act as mixed agonists-antagonists. Our work will serve as a proof-of-principle demonstration of the use of rapid kinetic resolution of TF degron models for a more precise characterization of the target specificity and efficacy of TF-directed inhibitors. Benchmarking of TF modulators against degron models in nascent transcriptomics assays should be considered as an important criterion in their functional characterization.

Disclosures No relevant conflicts of interest to declare.

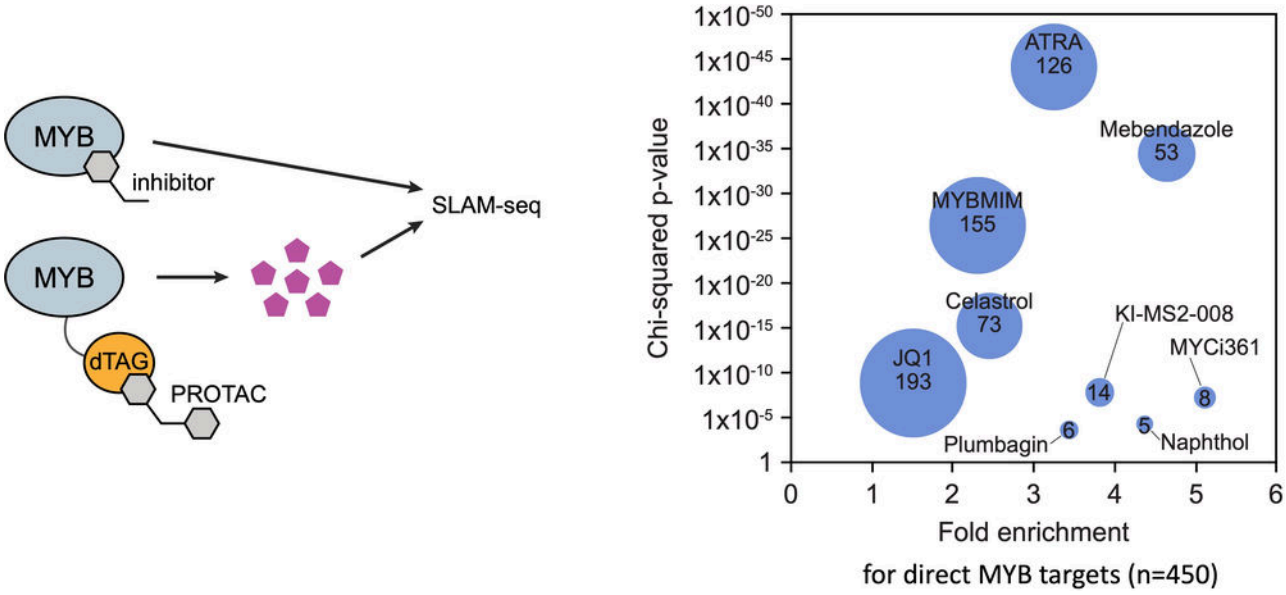

Figure 1

https://doi.org/10.1182/blood-2023-186841