

## The Myb-p300 Interaction Is a Novel Molecular Pharmacologic Target

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See related article by Uttarkar et al., p. 1276

There are large and growing numbers of molecularly targeted agents under study in oncology. Yet, the nature of the pathways targeted in the cancer clinic should be broadened (1). Success stories include the discoveries of antagonists that affect growth factor receptors and enzymes that are either overexpressed or mutated relative to their normal counterparts. These proteins function as oncogenic changes that drive the growth, survival, or abnormal differentiation state of a specific cancer. Clinical examples of this include HER2-targeted treatments of breast cancer (2), inhibitors of BRAF in melanoma (3), or antagonists of the epidermal growth factor receptor (EGFR) and its tyrosine kinase in lung cancer (4), to cite a few of many possible ones. However, most cancers diagnosed in patients do not harbor such alterations. Hence, other biochemical pathways that interfere with aberrant growth of cancers must be found.

One way to expand the spectrum of such targets is to disrupt protein-protein interactions. This is the case when cancers depend on them for their growth, maturation, or survival. Promising antineoplastic strategies would then be uncovered. Indeed, targets once considered intractable would become exposed. This is why the study by Uttarkar and colleagues (5) is noteworthy. They revealed a way to interfere with the oncogenic effects of one of the earliest known oncogenes, *Myb* (6). This oncogene was previously considered an unattractive pharmacologic target. They accomplished the feat of showing that Myb is amenable to interference by exploiting a critical c-Myb protein-protein interaction. This builds on prior work that showed Myb-dependent gene expression was inhibited by the sesquiterpene lactone mexicanin-1 (7).

Myb functions as a transcription factor via binding to its genomic binding site t/cAACT/gG (8). Myb also interacts with the coactivators CBP or p300 to regulate gene expression (9, 10). The KIX domain of p300 interacts with a LXXLL motif in the transactivation domain of c-Myb (11). More than 80 Myb target genes exist and are placed into three functional groups. These include housekeeping genes such as *MAT2A* and *GSTM1*, cell lineage and differentiation gene products as, for instance, *ELA2*, *MIM1*, *CD4*, and *PTCRA* as well as species involved in the carcinogenesis process such as *MYC*, *Cyclin A1*, *Cyclin E*, *KIT*, *BCL2*, *HSPA5*, and *GATA3* (12, 13).

Myb plays critical roles in lineage determination, stem and progenitor cell proliferation, as well as in controlling differentiation (6). It is a major species in determining hematopoietic lineage in lymphoid and erythroid cells (14). Immature or progenitor-like cells typically have high levels of Myb, whereas differentiated cells have low levels (15). Myb is a key regulator of the adult colonic crypt. Loss or repression of Myb in the colon results in reduced crypt size and proliferation, increased goblet cells, and decreased enterocytes as well as enteroendocrine cells (16).

Myb was initially identified as a retroviral oncogene (v-Myb) of avian myeloblastosis virus (17). It has vital functional roles in diverse malignancies. For example, Myb is highly expressed in hematopoietic progenitor cells, and its forced overexpression blocks differentiation and promotes leukemic cell transformation (15). Leukemic cells often depend on high basal c-Myb protein expression for their survival (18). Myb also has a major role in the development of several solid tumors. It is overexpressed in more than 80% of colorectal cancers (19). This expression profile predicts an unfavorable clinical outcome (20). Myb is detected in subsets of breast cancers and is associated with expression of the estrogen receptor- $\alpha$  (ER $\alpha$ ; ref. 21) in part because Myb is a direct ER $\alpha$  target (22). Myb oncogene addiction in leukemia and its expression in other cancers provided a rational basis for targeting Myb in cancer therapeutics.

Naphthol AS-E phosphate is an organophosphate. It was first used in histochemical experiments to quantify alkaline and acid phosphatase activities in polyacrylamide membrane model systems (23). Its biologic activity was found when naphthol AS-E phosphate was determined to disrupt the interaction between the CREB and CBP complex and attenuate target gene expression in response to exposure to a cAMP agonist (24). Naphthol AS-E phosphate is now shown to interfere with binding between the KID domain of CREB and the KIX domain of CBP (5). This intriguing work established that a small molecule is able to disrupt this protein-protein interaction in the nucleus. This interferes with subsequent signaling cascades. In turn, this establishes a biochemical basis for targeting interactions between Myb and p300, because the KIX domain of p300 complexes with Myb in hematopoietic cells (25).

Uttarkar and colleagues (5) used the bacterial autodisplay assay to discern an interaction between Myb and the KIX domain of p300. They built on this experimental approach by showing that naphthol AS-E phosphate antagonizes this association and by this represses Myb transcriptional activity. Using microscale thermophoresis, these investigators determined the dissociation constant for the Myb-KIX association ( $\sim 2.5$   $\mu\text{mol/L}$ ) and  $\text{IC}_{50}$  for inhibition of the Myb-KIX interaction by naphthol AS-E phosphate ( $\sim 30$   $\mu\text{mol/L}$ ). These findings were extended by studying the human myeloid leukemia cell lines HL60, U937, and NB4. These studies determined that treatment with naphthol AS-E phosphate is able to trigger differentiation and to reduce expression of c-Myc

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and Ada, which are Myb target genes. Apoptosis was also conferred by treatments of these leukemic cell lines. However, this was observed at a relatively high naphthol AS-E phosphate concentration ( $\sim 25 \mu\text{mol/L}$ ).

There are several notable aspects of this study. One is that the autodisplay assay is a powerful way to identify specific protein interactions. This technology takes advantage of *E. coli* bacteria to express recombinant proteins at high levels on the cell surface, which allows for functional readout of a desired protein interaction (26). This team used this assay to identify the Myb–KIX association and to measure the dissociation constant and  $\text{IC}_{50}$  of naphthol AS-E phosphate. A key aspect of this work was to confirm that it is possible to antagonize the Myb oncoprotein with a small molecule inhibitor (5). Although their study used the tool compound (naphthol AS-E phosphate), further work is needed to develop a clinical lead compound that would selectively inhibit Myb in the cancer clinic.

Some limitations in the current work could be addressed in future studies. For instance, the selectivity and specificity of naphthol AS-E phosphate for the Myb–KIX interaction need to be determined. Naphthol AS-E phosphate does disrupt interactions between this transcription factor and the KIX domain of CBP/p300. However, the current study does not exclude the possibility that naphthol AS-E phosphate effects on Myb target gene expression, differentiation, and apoptosis are conferred by mechanisms other than those affecting Myb–CRB or another transcription factor (CREB and c-Jun) interaction with the KIX domain of p300/CBP (27). In this regard, it is relevant that the Myb–CBP binding  $K_d$  is  $0.65 \mu\text{mol/L}$ , but the Myb–p300 binding is higher ( $K_d = 2.5 \mu\text{mol/L}$ ). Some antineoplastic activities of naphthol AS-E phosphate occur at high dosages, indicating that Myb–p300 off-target effects are potentially engaged.

Whether other KIX domain interactions are involved needs to be learned. Naphthol AS-E phosphate was shown to suppress

expression of Myb target genes and induce differentiation at concentrations as low as  $5 \mu\text{mol/L}$ , but apoptosis was not prominently detected until higher dosages were used (5). This underscores the possible off-target actions of this agent. Additional mechanistic studies would establish whether naphthol AS-E phosphate pharmacodynamic activities in myeloid leukemic cells depend on disrupting the Myb–p300 association. This can be accomplished by performing gain versus loss of Myb and/or p300 functional studies as a way to discern consequences on leukemic cell proliferation, apoptosis, and differentiation. The knowledge obtained from these analyses would help guide the development of agents with even greater ability than naphthol AS-E phosphate to antagonize Myb–p300 protein interactions.

In summary, naphthol AS-E phosphate disrupts interactions between Myb and the KIX domain of p300. The result of this includes induced myeloid leukemia cell differentiation and apoptosis. Taken together, the current work (5) underscores the value of identifying innovative molecular pharmacologic targets such as Myb. It is notable that Myb is a candidate therapeutic target in malignancies beyond leukemia. Future work should identify agents with more favorable properties than naphthol AS-E phosphate in antagonizing Myb activity. This would certainly broaden the scope of pharmacologic tools available to combat cancer.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

Conception and design: X. Liu, E. Dmitrovsky

Writing, review, and/or revision of the manuscript: X. Liu, K.A. Gold, E. Dmitrovsky

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