HAT Trick: p300, Small Molecule, Inhibitor

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p300 is an acetyltransferase that targets histone and nonhistone proteins for lysine acetylation. Based on a p300 structure, Bowers et al. (2010) have conducted an in silico screen for active site small molecule inhibitors of p300. We describe this important discovery and its potential therapeutic applications.

In human cells, a variety of structurally unrelated enzymes catalyze the transfer of acetyl groups from acetyl-coenzyme A to lysine residues present in acceptor proteins. As a posttranslational modification, acetylation can affect many aspects of protein function, including protein-protein interactions, enzymatic activity, subcellular localization, and protein stability. Acetylation was first discovered on histones and has been most studied in this context where it participates in the control of gene expression and influences biological processes as diverse as development, inflammation, metabolism, cancer, memory, and viral infections. The acetyltransferase enzymes were called histone acetyltransferases (HATs) until recently, when the name was changed to lysine acetyltransferases (KATs) to acknowledge a fast growing number of nonhistone substrates (Allis et al., 2007). These nonhistone substrates have significantly broadened the biological significance of HATs or KATs, and form the molecular basis for their diverse and vital role in biology.

p300 and its paralog CREB-binding protein (CBP), now called KAT3A and KAT3B, have more than 70 defined histone and nonhistone substrates (Wang et al., 2008). The number is presumably much larger since the two enzymes are known to interact with at least 400 cellular binding partners, making them two of the most connected proteins in the mammalian protein-protein interaction network (Bedford et al., 2010). Accordingly, both enzymes are indispensable during mammalian development: mice lacking p300 or CBP die early during development (E10.5). In humans, mutations in p300 or CBP are associated with Rubinstein-Taybi syndrome, a congenital developmental disorder characterized by distinct facial features, short stature, moderate to severe learning difficulties, and broad thumbs and first toes. Chromosomal translocations involving CBP or p300 are associated with leukemia, most notably the t(8;16)(p11;p13) translocation associated with a subtype of acute myeloid leukemia. This translocation fuses MOZ, a distinct HAT/KAT belonging to the MYST family of acetyltransferases, to the amino terminus of CBP. Since the fusion occurs in frame, excessive acetyltransferase activity of the fusion protein is credited with its oncogenic potential.

The broad substrate specificity of p300 is correlated with unique structural features of its acetyltransferase domain (Liu et al., 2008). The enzyme lacks a deep substrate-binding pocket, which is thought to prohibit formation of a stable ternary complex between enzyme, acetyl-coenzyme A, and substrate (Wang et al., 2008). The enzyme is thought to bind first to acetyl-coenzyme A and then transiently to the positively charged target lysine, which dissociates immediately after acetyl transfer. Substrate binding is mediated via "shallow" stretches of electronegative amino acids within the p300 acetyltransferase domain, which markedly differs from more neutral amino acids located at the corresponding positions in other HATs/KATs. This "hit-and-run" mechanism is unique to p300 and CBP, opening the door to the development of selective small-molecule inhibitors.

Despite its promiscuous substrate specificity, the catalytic activity of p300 is highly regulated in cells. The acetyltransferase activity is intrinsically weak but activated after autoacetylation of a basic activation loop located within the p300 acetyltransferase domain. This loop presumably covers the electronegative substrate-binding site under nonacetylated conditions but allows substrate access after autoacetylation (Wang et al., 2008). Interestingly, p300 autoacetylation occurs only transiently at gene promoters where transcription is initiating. Autoacetylation is counterbalanced by the activity of the NAD^+-dependent deacetylase SIRT2, which maintains p300 in a deacetylated and enzymatically less active state (Black et al., 2008). To ensure maximal enzymatic activation, p300 not only acetylates itself but also its inhibitor SIRT2, a process that inactivates SIRT2 and boosts p300 autoacetylation and catalytic activity (Han et al., 2008) (Figure 1).

Given the broad and important role of p300 in various biological processes, there is considerable interest in identifying p300 inhibitors and in testing their activities as potential therapeutic agents. Cole and colleagues reported the first inhibitor class for p300. A Lys-coenzyme A conjugate, designed as a bisubstrate inhibitor, functions as a potent (K_i 20 nM) and selective p300 inhibitor (Lau et al., 2000). Another coenzyme A conjugate with a histone H3 peptide was shown to function as a selective PCAF inhibitor (Lau et al., 2000). Lys-CoA was made cell permeable by attachment to a Tat peptide and was used in a number of studies, but its complexity has limited its further pharmacological development. Subsequent reports identified several natural products as p300 inhibitors including curcumin (isolated from turmeric), garcinol (garlic), and anacardic acid (cashew nut). Curcumin is a polyphenolic compound that has been used as a component of Indian Ayurvedic medicine. In vitro and animal studies have suggested that curcumin may have antitumor, antioxidant, antiarthritic, and anti-inflammatory properties. High throughput screens also led to the identification of several synthetic small

molecules that inhibit p300 but their selectivity and mechanisms of inhibition have not been fully elucidated. These limitations have restricted the exploration of the therapeutic potential of p300 as a drug target.

Bowers et al. (2010) now report the identification of a novel p300 inhibitor. They used the structure of the p300 HAT domain complexed with Lys-CoA for in silico screening (Liu et al., 2008). A library of 500,000 commercially available compounds was computationally docked into the Lys-CoA binding pocket. Out of 194 initial leads, 3 compounds were found to be relatively potent p300 inhibitors (K_i < 5 μM). One of these compounds, C646, a pyrazolone-furan, was highly selective against p300 in comparison with six other HATs and was shown to function as a reversible linear competitive inhibitor of p300 versus acetyl-coA (K_i = 400 nM). Mutation of Trp1466 (to Phe) in the catalytic active site of p300 induced a 7-fold decrease in affinity for C646, supporting the model that this inhibitor binds directly to the catalytic pocket of p300 and competes with its substrate.

Importantly, the p300 inhibitor C646 suppressed histone H3 and H4 acetylation in mouse fibroblast cell lines and suppressed the growth of melanoma and lung cancer cell lines in vitro. In contrast, a nontransformed murine cell line (NIH 3T3) was not inhibited by C646. Inhibition of transformed cell line growth was associated with a decreased histone acetylation and with a G1/S cell cycle arrest, consistent with p300 inhibition. These observations support the model that p300 inhibition represents a novel and promising therapeutic target for cancer.

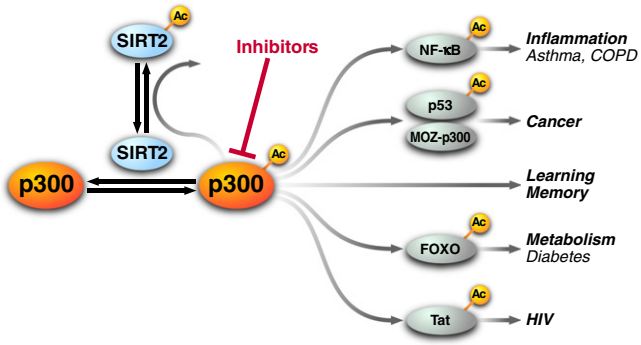

Figure 1. p300 Regulation and Its Nonhistone Substrates
p300 autoacetylation enhances its acetyltransferase activity. The NAD-dependent sirtuin SIRT2 deacetylates p300 and inactivates it, while p300 acetylation of SIRT2 inhibits its deacetylase activity. Several nonhistone substrates of p300 and their possible therapeutic implications are shown.

Much remains to be learned about p300. Although p300 and CBP share extensive sequence homology, they are not identical and have distinct biological activities. Future efforts in this field could focus on the development of p300 versus CBP selective inhibitors. In addition, several biological functions of p300 have been described that are independent of its acetyltransferase activity. For example, mutations within the p300 acetyltransferase domain have little effect on hematopoiesis. Notably, CBP and p300 both possess E4 polyubiquitin ligase activities, which may account for the acetylation-independent functions of these proteins (Grossman et al., 2003; Shi et al., 2009).

The development of selective p300 inhibitors represents a milestone in the field of protein acetylation. While inhibitors of histone/protein deacetylases (HDAC/KDAC) have been the focus of intense study (two HDAC inhibitors were recently approved by the Food and Drug Administration and are in the clinic), the field of HAT/KAT inhibitors has been somewhat lagging. The discovery of selective new p300 inhibitors should allow for more extensive and definitive testing of its potential as a therapeutic target in a number of important pathological conditions.

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