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Minireview

CBP/p300 acetyltransferase activity in hematologic malignancies

Ritika Dutta, Bruce Tiu, Kathleen M. Sakamoto *

Division of Hematology/Oncology, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA

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ABSTRACT

CREB binding protein (CBP) and p300 are critical regulators of hematopoiesis through both their transcriptional coactivator and acetyltransferase activities. Loss or mutation of CBP/p300 results in hematologic deficiencies in proliferation and differentiation as well as disruption of hematopoietic stem cell renewal and the microenvironment. Aberrant lysine acetylation mediated by CBP/p300 has recently been implicated in the genesis of multiple hematologic cancers. Understanding the effects of disrupting the acetyltransferase activity of CBP/p300 could pave the way for new therapeutic approaches to treat patients with these diseases.

Contents

1. CREB-binding protein structure and function

1.1. CBP gene and protein structure

CREB binding protein (CBP) is encoded by a 190 kb gene located on chromosome 16p13.3. Transcription yields a 7.3 kb mRNA, which is translated into a protein of 2442 amino acid residues in humans [1,2]. The major protein domains of CBP include: the nuclear receptor

* Corresponding author at: CCSR1215C, 269 Campus Drive, Stanford, CA 94305-5162, USA.
E-mail address: kmsakamo@stanford.edu (K.M. Sakamoto).

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interaction domain, the CREB-interacting kinase-inducible domain interacting (KIX) domain, three cysteine/histidine (CH) rich domains, a cyclin-dependent kinase inhibitor-reactive domain (CRD1) involved in cell cycle regulation and transcriptional suppression, a bromodomain (BRD) shown to recognize acetylated lysines, a 380 residue protein lysine acetyltransferase (KAT) domain, and a nuclear receptor coactivator binding domain (NCBD), also known as interferon binding domain (IBiD) [3–15] (Fig. 1). As a consequence of its many domains and large size, CBP serves as a scaffold and interprets molecular signals through its interactions with various proteins.

CBP and its homologue p300, two proteins closely related in structure and function, compose the p300/CBP coactivator family, which includes other proteins such as p270 [16]. CBP and p300 have a high degree of homology and their functions are largely redundant [17]. However, CBP/p300 can play distinct roles *in vivo*. Mice with a mutated *p300* allele with defective acetyltransferase activity display more severe heart, lung, and small intestine defects compared to their mutant *Cbp* counterparts [18], while $Cbp^{+/-}$ mice show craniofacial abnormalities and growth retardation [19,20]. Thus, CBP/p300 cannot compensate for each other completely, although they share many functionalities [21,22]. CBP and p300 play major roles in embryogenesis [23] [24,25], hematopoiesis [20,26], and myogenesis [27]. They are expressed in most cell types, including hematopoietic cells [20,26], germ cells [28, 29], neurons [30], myocytes [31], epithelial cells [32], hepatic cells [33], lung cells [34], osteoblasts [35], and pancreatic cells [36]. At the cellular level, they regulate diverse cellular processes such as proliferation [37,38], differentiation [20], and apoptosis [39] by integrating cellular signals and transcriptional regulation of target genes involved in these cellular processes.

The transcriptional integration and regulation of CBP/p300 is achieved through two different cellular functions: protein and histone lysine acetyltransferase activity and molecular scaffold function to link transcriptional complexes to basal transcriptional RNA polymerase machinery [16]. The ability of CBP/p300 to interact with a wide variety of proteins makes it a major regulator of key signaling pathways [15]. This review describes the role of the acetyltransferase activity of CBP/p300 in the regulation of cellular functions.

1.2. Lysine acetyltransferase activity

Originally characterized by its interaction with protein kinase A (PKA) phosphorylated CREB [40], CBP regulates gene expression by recruiting components needed for transcriptional machinery and altering chromatin structure by acetylating histones and other proteins through KAT activity. The histone acetyltransferase (HAT) function of CBP/p300 is a subset of broader lysine acetyltransferase (KAT) activity of CBP/p300. CBP possesses intrinsic KAT activity capable of acetylating multiple lysine residues on core histones [41]. Additionally, CBP can recruit P/CAF (p300/CBP-associated factor) to promoters of genes [42]. P/CAF complexes with CBP/p300 and has intrinsic acetyltransferase

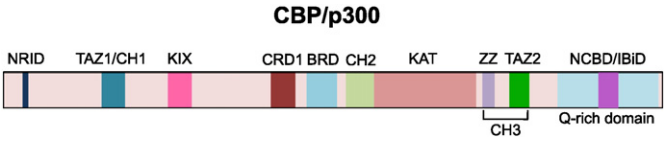

Fig. 1. Domain structure of CBP/p300. A schematic representation of the main protein interaction domains of CBP/p300, including the nuclear receptor interaction domain (NRID), the cysteine-histidine rich domain 1 (CH1) (also known as the transcriptional adaptor zinc finger 1 domain, TAZ1), the CREB and c-Myb interacting KIX domain, the cyclin-dependent kinase inhibitor-reactive domain (CRD1), the bromodomain (BRD), another cysteine-histidine rich domain (CH2) which contains a plant homeodomain, the lysine acetyltransferase domain (KAT), a third cysteine-histidine rich domain (CH3) containing a small zinc binding domain (ZZ) and transcriptional adaptor zinc finger 2 domain (TAZ2), and a glutamine (Q)-rich region encompassing a nuclear receptor coactivator binding domain (NCBD) (also known as the interferon-binding domain, IBiD).

activity that may stimulate activation of factors associated with CBP through promoter-specific acetylation [43].

Bannister and Kouzarides [42] first characterized the intrinsic KAT activity of CBP, located in residues 1099–1758. Histone acetyltransferases function by transferring an acetyl group to lysine residues on histones, neutralizing their positive charge and weakening their interactions with DNA. Histone acetylation has been shown to regulate transcriptional activity by reorganizing higher-order chromatin structures and promoting an 'unpacked' structure that is more accessible to transcriptional machinery [44]. CBP and p300 form the KAT3 family, defined by their ability to catalyze transcription-related acetylation events [45]. Unlike other KATs, the structure and kinetics of CBP/p300 suggest that the proteins utilize a Theorell-Chance catalytic mechanism, in which no stable ternary complex is formed. The unique structure and enzymatic mechanism of the CBP/p300 KAT domain explain the specificity of its substrates [46]. Although CBP/p300 have overlapping substrate specificities in histone H3 and H4, the acetyltransferase activity of each enzyme shows different substrate specificities depending on the availability of histone or acetyl-CoA [47]. This suggests that differences in substrate specificity may emerge that are dependent on histones falling off newly transcribed or replicated strands of DNA, or in metabolically dysregulated cancer cells.

Genome-wide distribution studies of acetyltransferases through ChIP-Seq analysis indicated that CBP/p300 are highly enriched in the promoters and enhancers of active genes [48], consistent with CBP/p300 KAT activity regulating gene transcription. Specifically, deletion of CBP/p300 reveals that their KAT activities are required for acetylation of H3K18 and H3K27, subsequent recruitment of RNA polymerase II, and gene expression in mouse embryonic fibroblast cells [49]. Our concept of the transcriptional role of CBP/p300 has recently expanded with the discovery that they are part of a set of proteins greatly enriched on super-enhancers [50]. This implicates CBP/p300 as specifying normal cell fate through super-enhancer function. Moreover, the aberrant formation of super-enhancers, critical drivers of oncogene expression, occurs during tumorigenesis [50]. This includes super-enhancer driven c-myc expression in multiple cancer types and the over-expression of multiple genes involved in every hallmark of cancer [50–52]. Targeting super-enhancers shows great promise as a cancer therapeutic, as cancer cells are disproportionately sensitive to the loss of super-enhancers and their coactivators. Thus, many cancer indications are expected to be targeted by inhibition of single or multiple super-enhancer drivers, including CBP/p300.

Importantly, the KAT domain of CBP has many non-histone substrates. Thus, CBP can additionally mediate protein-protein interactions involved in signal transduction through non-histone acetylation, which can have either an inhibitory or stimulatory effect on gene transcription [65]. CBP/p300 has been shown to acetylate many transcription factors, such as p53 [53], CREB [54], E2F-1, E2F-2 and E2F-3 [55], MYB [56], MyoD [57], GATA-1 [58], and NF-Y [59]. Structural analyses have revealed that the bromodomain binds to acetylated lysine peptides [60, 61]. Around one-hundred proteins have been reported to be acetylated substrates of CBP/p300, and several acetylated proteins including STAT3 and p53 bind to the bromodomain of CBP/p300 [62], suggesting that lysine acetylation of proteins by the KAT domain plays a critical role in facilitating the association of proteins to CBP/p300 through the bromodomain. Acetylation of these transcription factors increases DNA binding or association with CBP/p300 in order to facilitate expression of target genes. Conversely, CBP/p300 also attenuates FoxO-mediated transcriptional activity by acetylating its DNA-binding domain [63].

CBP KAT activity is tightly regulated during cell cycle progression. Phosphorylation of CBP, discussed below, significantly increases its KAT activity, which peaks at the G1-S transition and is necessary for E2F-dependent transcription and S-phase entry [38]. Because Cyclin-E/CDK2 activity correlates with elevated CBP KAT activity, CDK2 has been suggested to play a role in regulating CBP phosphorylation. Further supporting this notion, inhibitors of the Cyclin-E/CDK2 complex prevent