Targeting p300 Addiction in CBP-Deficient Cancers Causes Synthetic Lethality by Apoptotic Cell Death due to Abrogation of MYC Expression

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ABSTRACT

Loss-of-function mutations in the CBP/CREBBP gene, which encodes a histone acetyltransferase (HAT), are present in a variety of human tumors, including lung, bladder, gastric, and hematopoietic cancers. Consequently, development of a molecular targeting method capable of specifically killing CBP-deficient cancer cells would greatly improve cancer therapy. Functional screening of synthetic-lethal genes in CBP-deficient cancers identified the CBP paralog p300/EP300. Ablation of p300 in CBP-knockout and CBP-deficient cancer cells induced G1–S cell-cycle arrest, followed by apoptosis. Genome-wide gene expression analysis revealed that MYC is a major factor responsible for the synthetic lethality. Indeed, p300 ablation in CBP-deficient cells caused down-regulation of MYC expression via reduction of histone acetylation in its promoter, and this lethality was rescued by exogenous MYC expression. The p300-HAT inhibitor C646 specifically suppressed the growth of CBP-deficient lung and hematopoietic cancer cells in vitro and in vivo; thus p300 is a promising therapeutic target for treatment of CBP-deficient cancers.

SIGNIFICANCE: Targeting synthetic-lethal partners of genes mutated in cancer holds great promise for treating patients without activating driver gene alterations. Here, we propose a “synthetic lethal-based therapeutic strategy” for CBP-deficient cancers by inhibition of the p300 HAT activity. Patients with CBP-deficient cancers could benefit from therapy using p300-HAT inhibitors. Cancer Discov; 6(4); 1–16. ©2015 AACR.

See related commentary by Kadoch, p. xxx.

INTRODUCTION

Current approaches for precision medicine for treatment of human cancer depend largely on targeting activated protein kinases using specific inhibitors or antibodies. Kinase activation in cancer is caused by genetic aberrations, such as the ABL gene fusion in chronic myelogenous leukemia, the ERBB2/HER2 amplification in breast and gastric cancers, and EGF mutations and the ALK fusion in non–small cell lung carcinoma (1, 2). Such genetic aberrations constitute a specific vulnerability of cancer cells due to “oncogene addiction,” a state in which inhibition of the aberrant proteins causes death or growth arrest of cancer cells. Recent studies by our group and other groups identified other kinase gene aberrations as promising therapeutic targets: FGFR family gene amplifications and fusions (3–6), AKT Family gene mutations and fusions (7, 8), and fusions of RET, ROS1, and NTRK1 (9–13). However, these studies also revealed that only a small fraction of cancers carry such druggable aberrations in kinase genes; therefore, to further advance precision cancer medicine, it is essential to identify non-kinase gene aberrations that can be targeted in the clinic.

The most commonly mutated genes in human cancers encode chromatin modifiers (14, 15). Genes that encode components of the SWI/SNF chromatin remodeling complex, such as SMARCA4/BRG1 and ARID1A/BAF250A, are frequently inactivated by protein truncation mutations and gross gene deletions (14, 16, 17). Notably, such chromatin modifier genes work with and complement their structural and/or functional paralogs; consequently, cancer cells with deficiencies in chromatin modifiers are predicted to exhibit addiction (analogous to oncogene addiction) to the remaining intact paralogs. Based on this fact, we recently proposed a novel therapeutic strategy, “paralog targeting,” for use against cancer cells with such gene deficiencies (18, 19). In this approach, the remaining intact paralog of a deficient chromatin modifier gene is the target of therapeutic inhibition. We first demonstrated the feasibility of this strategy by achieving specific killing of SMARCA4/BRG1-deficient cancers through inhibition of the SMARCA4 paralog SMARCA2/BRM-ATPase (19). These findings were subsequently supported by several studies, including some that employed genome-wide RNA interference scanning (20–22). Recently, this concept has been extended to ARID1A-deficient cancers (23). Based on successful precision medicine approaches that have target-activated oncogene products, paralog targeting strategies must meet the following criteria to be successfully translated to the clinic. First, cancer cells with a gene deficiency must exhibit greater reliance on the remaining paralogs than cells without that deficiency, including noncancerous cells. Second, the molecular mechanism underlying the addiction must be proven. Third, existing inhibitors of the remaining paralog must exert a specific therapeutic effect on cancer cells harboring a deficiency in a chromatin modifier.

CBP/CREBBP (CREB-binding protein) and p300/EP300 are chromatin modifier proteins that acetylate two lysine (K) residues on histone H3, K18 and K27 (24, 25). CBP- and p300-mediated histone acetylation at gene promoter/enhancer
regions collaborates with SWI/SNF complexes to facilitate remodeling of chromatin into a relaxed state (26–28), allowing access by RNA polymerase II (29). We showed that approximately 10% to 15% of non–small cell and small cell lung cancers harbor loss-of-function aberrations in the CBP gene (30, 31). Recent genome-wide sequencing studies reveal that such aberrations are also prevalent in multiple types of human cancer, including lymphoma (29%–33%), leukemia (18%), and bladder cancer (15%–27%; refs. 22, 32–37). In addition, the recent detection in lung cancer of deleterious mutations of KAT6B, which encodes another histone acetyltransferase (HAT), expands the known role of HAT gene aberrations in carcinogenesis (38). Recurrent missense mutations in CBP tend to cluster around the region encoding the HAT domain. In particular, mutations affecting the amino-acid residues p.Gly1411, p.Trp1472, and p.His1487, which ablate HAT and/or transcriptional coactivation activity (30, 35), are frequently observed. In addition, gross deletions and protein-truncating mutations are often detected (6, 30, 35, 39). Development of therapeutic strategies for specific killing of CBP-deficient cancer cells will significantly advance precision cancer medicine. In this study, a functional synthetic-lethal screen revealed that CBP-deficient cancer cells were killed by suppression of the paralog p300. Here, we describe a paralog targeting strategy that exploits deficiency of CBP in human cancers and satisfies the aforementioned criteria for successful translation into the clinic.

RESULTS

Identification of p300 as a Specific Synthetic-Lethal Gene in CBP-Deficient Cancer Cells

To identify synthetic-lethal partner genes in CBP-mutated cancer cells, we screened an siRNA library that targets genes involved in chromatin regulation, chromatin remodeling, histone modification, and histone marker recognition. To isolate genes specifically required for growth of CBP-mutated human cancer cells, we initially performed the screen in the two noncancerous lung cell lines, HFL1 and MRC5; a CBP wild-type (WT) lung cancer cell line, A549; and a CBP-mutated (homozygous deleted) lung cancer cell line, LK2. Six genes were identified as candidate lethal genes specifically in CBP-mutated cancer cells (Fig. 1A; Supplementary Table S1). We validated these six candidates using another set of human lung cancer cells: parental H1299 cells with intact CBP; H1299 cells with artificial homozygous CBP-knockout (KO), designated H1299 2G2; and a CBP-deficient lung cancer cell line, LK2. The top-hit gene in this analysis was p300, whose knockdown exerted antiproliferative and antisurvival effects specifically in CBP-KO and CBP-deficient cells (Fig. 1B; Supplementary Fig. S1A–S1C). No such effects of p300 depletion were observed in three noncancerous cell lines: MRC5, HEK293T, and RPE1-hTERT (Supplementary Fig. S1C and S1D). These data indicate that p300 is an essential factor specifically required for growth and survival of CBP-mutated cancer cells.

Synthetic Lethality of CBP and p300 Is the Result of G1 Arrest and Apoptosis

CBP and p300 are paralogous HAT proteins with highly similar amino-acid sequences and some overlapping functions (29, 40). Thus, identification of p300 as a lethal gene in the context of CBP deficiency indicates that p300 protein can replace some essential functions of CBP in CBP-deficient cancer cells. This relationship is predicted to create a specific vulnerability, i.e., more severe functional loss of CBP should lead to a more pronounced dependency on p300. To examine the relationship between CBP and p300 in the same genetic background, we established p300-KO cells in the H1299 lung cancer cell line, as well as in the CBP-KO cells used above (Supplementary Fig. S1E). Indeed, siRNA-mediated p300-knockdown (KD) caused marked suppression of growth and survival in CBP-KO cells, but had little or no impact in CBP WT cells (Fig. 1C and D and Supplementary Fig. S1F and S1G). Similarly, CBP-KD caused marked suppression of growth and survival in p300-KO cells, but not in p300 WT cells (Fig. 1C and E and Supplementary Fig. S1H and S1I). Next, we investigated the impact on the cell cycle and apoptotic cell death. In CBP-KO cells, depletion of p300 initially caused G1 arrest, and then gradually induced apoptotic cell death (Fig. 1F–H). Similarly, depletion of CBP in p300-KO cells caused G1 arrest and subsequent apoptotic cell death (Fig. 1I, J). Double depletion of CBP and p300 in cells proficient for both genes caused transient growth suppression but did not affect sustainable colony formation; this observation was further supported by the induction of transient G1 arrest, but not apoptosis, under these conditions (Supplementary Fig. S1J–S1N). These data indicated that lethality due to depletion of CBP and p300 is caused by apoptotic cell death, and that this effect is specific to cells lacking the p300 and CBP genes, respectively.

Deregulation of MYC Transcription by p300-KD in CBP-Deficient Cells

Next, we performed genome-wide expression profiling analysis to define the genes underlying the synthetic-lethal relationship between CBP and p300. We identified 1,936 genes whose expression levels changed >2-fold upon p300-KD in CBP-KO cells, and upon CBP-KD in p300-KO cells, but not upon either KD alone in WT cells (Fig. 2A). Pathway analysis revealed that these genes were significantly (P < 0.001) enriched in 13 functional pathways, including those related to G1–S cell-cycle control (ranks 1–4) and apoptosis (rank 9), consistent with the phenotypes of G1 arrest and apoptosis observed following p300 depletion in CBP-KO cells or CBP depletion in p300-KO cells (Fig. 2A and Supplementary Table S2). Notably, the MYC oncogene was the gene that appeared most frequently in these 13 functional pathways (Fig. 2A; Supplementary Fig. S2A and Supplementary Table S3). Quantitative RT-PCR confirmed that the MYC mRNA level was reduced upon p300 depletion in CBP-KO cells or upon CBP depletion in p300-KO cells (Fig. 2B). Consistent with this, expression of MYC target genes, such as G1–S cyclins (Cyclin D1/CCND1 and Cyclin A2/CCNA2), cyclin-dependent kinases (CDK4 and CDK6), and an antiapoptotic factor (Survivin/BIRC5), was also reduced in CBP-KO cancer cells (Supplementary Fig. S2B). Concordantly, levels of MYC proteins were specifically reduced in CBP-KO cells, but not in CBP WT cells, upon p300 depletion (Fig. 2C). Cell growth and MYC expression in CBP-KO cells in which endogenous p300 was depleted by RNA interference targeting the 3′-untranslated region (UTR) of p300 was partially rescued by
Figure 1. Synthetic-lethal screening of chromatin modifier genes in CBP-deficient cancer cells. A, high-throughput screening to identify genes for which depletion is specifically lethal in CBP-deficient cancer cells. Noncancerous cells (HFL1 and MRC5), CBP WT cancer cells (A549), and CBP-deficient cancer (LK2) cells were seeded in 96-well plates and then transfected with siRNAs in duplicate. Each transfection plate contained 138 siRNAs targeting genes related to chromatin regulation; a pool of three different siRNAs targeted each gene. Cell viability was assessed 5 days after siRNA transfection. The screen identified six genes for which siRNA-mediated KD had little (≤35% inhibition) effect on cell viability in noncancerous cells and CBP WT cancer cells, but a marked (>65% inhibition) effect in CBP-deficient LK2 cancer cells. B, schematic time course of FACS analysis for examination of cell-cycle profile and apoptosis. C–E, H1299 parental (CBP WT), H1299 CBP-knockout (CBP-KO), and H1299 CBP-mutant (CBPmut) cells were transfected with siRNAs for 48 hours, and then assayed for growth or colony formation. The relative proliferating ratio or surviving fraction of si300-treated cells 5 days or 12 days after reseeding, respectively, is expressed as a heat-map plot of the percentage of H1299, CBP-KO H1299 2G2, and CBP-deficient LK2 cells transfected with siRNAs that proliferated or survived, relative to the corresponding percentage in cells transfected with nontargeting siRNA (siNT). C–E, inhibition of cell growth by depletion of p300 or CBP. F, schematic time course of colony formation assay. Cell growth assay and colony formation assay were performed using siNT and/or p300-knockout (KO) cells transfected with siRNA (siNT, si300 D1, or siCBP D2) for 48 hours, and then assayed for colony formation. The relative surviving fraction of siRNA-treated cells at 0 to 7 days or 12 days after reseeding, respectively, was expressed as a percentage of cells transfected with targeting siRNAs that survived, relative to the corresponding percentage in cells transfected with nontargeting siRNA. The numbers and sizes of colonies formed by cells in which CBP and/or p300 were depleted are shown in the panels at right. Data, means ± SD. F–J, increase in the proportion of G<sub>1</sub> phase and apoptotic cells upon depletion of p300 or CBP. F, schematic time course of FACS analysis for examination of cell-cycle profile and apoptosis. G–J, cancer cells (G, H, H1299 CBP-KO 2G2; I, J, H1299 p300-KO #23) were transfected with siRNA (siNT, si300 D1, or siCBP D2) for 48 hours. Two to eight days after reseeding, cell-cycle profiles and proportions of Annexin V-positive apoptotic cells were assessed by flow cytometry. Data, means ± SD.
MYC is a key determinant of cancer cell survival under p300- or CBP-depletion in CBP-KO or p300-KO cells, respectively. A genome-wide gene expression analysis identified MYC as the gene most strongly associated with the observed synthetic lethality. A Benno analysis identified 1,936 genes that exhibited significant changes in expression specifically upon p300 depletion in H1299 CBP-KO cells and CBP depletion in H1299 p300-KO cells. WikiPathways database analysis identified the 13 top pathways (<1,936 genes) that exhibited significant changes in expression specifically upon p300 depletion in H1299 KO cells by p300 depletion. CBP β-KO suppression of proliferation of H1299 p300-KO cells was rescued by exogenous expression of WT p300, but not HA T-defective p300. H1299 CBP-KO cells with or without stable exogenous CBP expression profiles of H1299 MYC, E2F1, by p300 depletion in CBP-KO cells. Forty-eight hours later (day 0), cells were harvested and subjected to quantitative RT-PCR analysis and immunoblot analysis. The relative changes in gene expression relative to siNT are shown. Data, means ± SD. Immunoblot analysis of p300, CBP/MYC, and β-actin (loading control) (C). D, suppression of proliferation of H1299 CBP-KO cells by p300 depletion was rescues by exogenous expression of WT p300, but not HAT-defective p300. H1299 CBP-KO cells were transfected with a plasmid expressing p300 or HAT-defective p300 (HAT′)-cDNAs. Twenty-four hours after transfection, the cells were further transfected with siNT, si300 [D1], or siCBP [D2]. After 48 hours, the cells were harvested and subjected to quantitative RT-PCR analysis and immunoblot analysis. The relative changes in gene expression relative to siNT are shown. Data, means ± SD. Vec, vector.

Figure 2. MYC is a key determinant of cancer cell survival under p300- or CBP-depletion in CBP-KO or p300-KO cells, respectively. A, genome-wide gene expression analysis identified MYC as the gene most strongly associated with the observed synthetic lethality. A Benno analysis identified 1,936 genes that exhibited significant changes in expression specifically upon p300 depletion in H1299 CBP-KO cells and CBP depletion in H1299 p300-KO cells. WikiPathways database analysis identified the 13 top pathways (<1,936 genes) that exhibited significant changes in expression specifically upon p300 depletion in H1299 KO cells by p300 depletion. CBP β-KO suppression of proliferation of H1299 p300-KO cells was rescued by exogenous expression of WT p300, but not HA T-defective p300. H1299 CBP-KO cells with or without stable exogenous CBP expression profiles of H1299 MYC, E2F1, by p300 depletion in CBP-KO cells. Forty-eight hours later (day 0), cells were harvested and subjected to quantitative RT-PCR analysis and immunoblot analysis. The relative changes in gene expression relative to siNT are shown. Data, means ± SD. Immunoblot analysis of p300, CBP/MYC, and β-actin (loading control) (C). D, suppression of proliferation of H1299 CBP-KO cells by p300 depletion was rescues by exogenous expression of WT p300, but not HAT-defective p300. H1299 CBP-KO cells were transfected with a plasmid expressing p300 or HAT-defective p300 (HAT′)-cDNAs. Twenty-four hours after transfection, the cells were further transfected with siNT, si300 [D1], or siCBP [D2]. After 48 hours, the cells were harvested and subjected to quantitative RT-PCR analysis and immunoblot analysis. The relative changes in gene expression relative to siNT are shown. Data, means ± SD. Vec, vector.

Exogenous expression of CCND1 and CDC45, the two genes appearing most frequently (other than MYC) in the 13 functional pathways (Fig. 2A), did not rescue the suppression of H1299 CBP-KO cell growth by p300 depletion (Fig. 2E). On the other hand, downregulation of these two genes and other top-ranked genes, E2F2 and E2F1, by p300 depletion in CBP-KO cells was rescues by exogenous MYC expression (Supplementary Fig. S2G), suggesting that MYC plays a key role in the lethality of CBP-deficient cells by p300 depletion. MYC protein is activated by dimerization with MAX protein (41). In fact, suppression of H1299 CBP-KO cell growth by p300
depletion was partially rescued by exogenous MAX expression (Supplementary Fig. S2H), indicating that deficiency of the MYC–MAX complex underlies the lethality of p300 depletion in CBP-deficient cells. Taken together, these data suggest that the vulnerability of CBP-KO cells is at least partially due to p300 HAT–dependent expression of MYC.

**p300 Depletion–Mediated Dysregulation of Chromatin Modification Causes Downregulation of MYC**

Next, we investigated the molecular mechanisms of MYC transcriptional repression in CBP-deficient cells following p300 depletion. Chromatin immunoprecipitation (ChIP) assays revealed that p300 and CBP localized to enhancer, promoter, and exon regions around the transcription start site (TSS) of the MYC gene in CBP-proficient, CBP-mutant, and CBP-KO cells (Fig. 3A and B and Supplementary Fig. S3). Acetylation at H3K18 and H3K27, which are redundantly acetylated by p300 and CBP (24), was abundant in the promoter region (Fig. 3C). p300 depletion in CBP-KO cells decreased the occupancy of acetylated H3K18 and H3K27 in the promoter region relative to that in CBP-proficient cells (Fig. 3C). Accordingly, recruitment of RNA polymerase II (RNAPII) to the TSS was markedly reduced upon p300 depletion in CBP-KO cells, and the phosphorylation of serine 5 of RNAPII, which is a marker of readiness for transcriptional initiation, was more impaired by depletion of p300 in CBP-KO cells than in CBP-proficient cells (Fig. 3C). Therefore, both p300 and CBP redundantly acetylate histones within the MYC gene locus, allowing the transcription of MYC. However, in CBP-KO cells, MYC expression is largely dependent on histone acetylation by p300 and is consequently repressed by p300 depletion.

**Addiction to p300 Due to Deleterious CBP Gene Aberrations**

We next investigated whether the proliferation and survival of other CBP-mutated cancer cell lines depends on p300. siRNA-mediated p300 depletion impaired both the survival and proliferation of cancer cells carrying missense CBP mutations with highly deleterious affect HAT activity (H520, H1703, and TE10) or nonsense mutations, resulting in truncation of the CBP protein (TE8 and LK2; Fig. 4A for survival; Supplementary Fig. S4A and S4B for cell growth). However,
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B

Cell cycle profile

C

Apoptosis

D

Fold change of Annexin V+ cells

E

Relative expression of EP300 and MYC mRNA

F

ChIP-seq analysis

G

Tumor volume over time

H

Tumor volume over time

I

Relative tumor weight
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p300 depletion had no apparent effect on cancer cells carrying missense CBP mutations with low or medium impacts on the HAT domain (LoVo, H322, H2009, H1048, H2087, and KM12C), or on CBP WT cells, regardless of p300 mutation (Fig. 4A for survival; Supplementary Fig. S4A and S4B for cell growth). Similar results were also obtained by depleting p300 with siRNAs targeting different sites, and also by constitutive expression of a lentivirally encoded shRNA (Supplementary Fig. S4C–S4E). Taken together, these data demonstrate that cancer cells that are CBP deficient due to loss-of-function mutations or deleterious aberrations are addicted to p300.

As in the case of the CBP-KO cells described above, the apoptotic fraction specifically increased in CBP-deficient cancer cells upon p300 depletion (Fig. 4B and C). These CBP-deficient cancer cells underwent apoptosis associated with PARP cleavage, but did not exhibit senescence or autophagy (Supplementary Fig. S4F). Expression of MYC mRNA and MYC protein was specifically reduced by p300 depletion in CBP-deficient cancer cells (Fig. 4D and E). Growth suppression by p300 depletion in CBP-deficient LK2 cancer cells was partially rescued by exogenous MYC expression (Supplementary Fig. S4G). In addition, in CBP-deficient cancer cells, p300 preferentially localized to the promoter region of the MYC gene, and p300 depletion impaired histone acetylation and RNAPII localization in that region (Fig. 4F). Therefore, in CBP-deficient cancer cells, p300-dependent MYC expression is essential for cell survival.

Therapeutic Potential of p300 Inhibition against CBP-Deficient Cancers

Next, using a mouse xenograft model, we investigated the in vivo therapeutic potential of p300 inhibition against CBP-deficient cancers. For these experiments, we established CBP WT H1299 and CBP-deficient LK2 lung cancer cells in which expression of p300 could be conditionally knocked down with doxycycline (Supplementary Fig. S4H). shRNA-mediated p300 depletion in CBP-deficient LK2 lung cancer cells, but not in CBP WT H1299 cancer cells, led to a reduction of growth and survival and an increase in the rate of apoptosis in vitro (Supplementary Fig. S4I–S4K). H1299 and LK2 lung cancer cells carrying conditionally expressed nontargeting (shNT) or p300-targeting (shp300) shRNAs were injected into the flanks of immune-deficient mice. When mice were fed doxycycline immediately after injection to induce p300 depletion, growth of LK2-shp300 xenografts was significantly suppressed, whereas the growth of LK2-shNT, H1299-shNT, and H1299-shp300 xenografts was unaffected (Fig. 4G; Supplementary Fig. S4L). When mice with engrafted tumors derived from CBP-deficient LK2 lung cancer cells were fed doxycycline to induce p300 depletion, the tumor growth and weight of LK2-shp300 xenografts, but not LK2-shNT xenografts, was significantly impaired (Fig. 4H and I and Supplementary Fig. S4M). Growth suppression of LK2 cells by p300 depletion was also observed in a mouse orthotopic model, in which lung cancer cells were implanted into the thoracic cavities of mice (Supplementary Fig. S4N). These data demonstrate the in vivo therapeutic potential of p300 inhibition against CBP-deficient lung cancers.

Sensitivity of CBP-Deficient Lung Cancer Cell to an Existing p300 HAT Inhibitor

To determine whether inhibition of p300 HAT activity is viable as a therapeutic strategy against cancer, we examined the ability of an existing specific inhibitor of p300 HAT, C646 (42), to suppress the growth of CBP-deficient lung cancer cells. C646 reduced survival in lung cancer cells carrying deleterious CBP mutations (H1703, H520, and LK2) to a greater extent than in CBP WT cells (A549, H1299, and H157) or cells harboring low-impact mutations (H322; Fig. 5A and Supplementary Fig. S5A). As in the case of p300-KD, C646 treatment led to a marked increase in the apoptotic fraction specifically in CBP-deficient cancer cells (Fig. 5B and Supplementary Fig. S5B). Accordingly, the level of MYC expression was specifically reduced in CBP-deficient cells (Fig. 5C and D and Supplementary Fig. S5C and S5D). In addition, in CBP-deficient cancer cells, localization of acetylated histone H3 and RNAPII in the promoter region of the MYC locus was impaired following treatment with C646, concomitant with a slight decrease in global acetylation levels of H3K18 and H3K27 (Fig. 5D and Supplementary Fig. S5D). Finally, we examined the therapeutic potential of C646 in a mouse xenograft model. C646 treatment led to a significant reduction in the growth suppression of LK2 xenografts, but not LK2-shNT xenografts, was significantly impaired (Fig. 4G; Supplementary Fig. S4L). When mice with engrafted tumors derived from CBP-deficient LK2 lung cancer cells were fed doxycycline immediately after injection to induce p300 depletion, the tumor growth and weight of LK2-shp300 xenografts, but not LK2-shNT xenografts, was significantly impaired (Fig. 4H and I and Supplementary Fig. S4M). Growth suppression of LK2 cells by p300 depletion was also observed in a mouse orthotopic model, in which lung cancer cells were implanted into the thoracic cavities of mice (Supplementary Fig. S4N). These data demonstrate the in vivo therapeutic potential of p300 inhibition against CBP-deficient lung cancers.

Figure 4. p300 depletion is lethal in cancer cells harboring loss-of-function mutations in CBP. A, synthetic-lethal effects assessed by colony formation assay. Data regarding missense and deleterious (Del) mutations were obtained from the CBioPortal and COSMIC databases. Functional impact values (neutral, low, medium, and high) of missense mutations were obtained from the MutationAssessor database. Cells were transfected with siRNA (shNT or shp300 Dp) for 48 hours and then assayed for colony formation. The surviving fraction of si300-treated cells after reseeding is expressed as the percentage of cells transfected with targeting siRNAs that survived, relative to the corresponding percentage of cells transfected with nontargeting siRNA. Data, means ± SD. B–E, increase in the proportions of G1 phase and apoptotic cells following depletion of p300 in CBP-deficient cancer cell lines. H157 and SQ5 (CBP WT) cells and H1703 and LK2 (CBP-deficient) cells were transfected with siRNA (shNT, shp300 Dp) for 48 hours. 96 hours after reseeding, the cells were subjected to flow cytometry to determine the cell-cycle profile (B) and the proportion of Annexin V–positive apoptotic cells (C). Immunoblot analysis (D) and quantitative RT-PCR analysis (E) 48 hours after siRNA-mediated p300 depletion of CBP WT and CBP-mutant cancer cells. Immunoblot analysis was performed using antibodies against p300, MYC, and β-actin (loading control). Expression levels of p300 and MYC were normalized against the level of GAPDH mRNA in the same samples. Data, means ± SD. F, suppression of transcriptional initiation of the MYC gene by impairment of histone acetylation upon p300 depletion in CBP-deficient LK2 lung cancer cells. Locations of the PCR amplicons in the MYC locus used for the ChiP assay (upper panel). The transcription start site (TSS) and promoter region (Pro) are indicated. Localization of p300 proteins at TSS and Pro in the MYC locus (left panel). Acetylation of Histone H3K27 (middle) and localization of RNA polymerase II (RNAPII; right) in the MYC locus 48 hours after transfection with shNT or shp300 Dp. G, suppression of in vivo growth of tumor cells by p300 depletion immediately after injection. H1299-shp300 and LK2-shp300 cells were implanted subcutaneously into BALB/c- nu/nu mice, and the mice were randomly divided into two groups: one group was fed a diet containing doxycycline (Dox+), and the other was fed a control diet (Dox–). Tumor volumes for each group are shown. H and I, suppression of in vivo growth of tumor cells by p300 depletion allowed to engraft after injection. LK2-shp300 cells were implanted subcutaneously into BALB/c-nu/nu mice. When the tumors reached more than 200 mm³, the mice were randomly divided into two groups and fed a diet containing doxycycline (Dox+) or a control diet (Dox–). Changes in tumor volume in both groups (H). Tumor weights at the time of sacrifice in both groups (I). Asterisks indicate significant differences in tumor volume or weight between doxycycline-fed and control mice (P < 0.05; Student t test). Data, means ± SE.
Figure 5. Response of CBP-deficient lung cancer cells to the p300 HAT inhibitor C646. A, survival of CBP-deficient cancer cells after treatment with C646. Cells were subjected to a colony formation assay in the presence or absence of 15 μmol/L C646. Surviving C646-treated cells are shown as a percentage of nontreated cells. Data, means ± SD. B, induction of apoptosis by C646 treatment. CBP WT H157 and CBP-deficient LK2 cells were treated with C646 (0 μmol/L, C646−) or 15 μmol/L (C646+) for 48 hours, harvested, and subjected to immunoblot analysis with antibodies against MYC, acetylated histone H3K18 and H3K27 relative to the level in nontreated cells. Data, means ± SD. C, expression of the MYC mRNA in cells treated with C646. CBP WT H157 and CBP-deficient LK2 cells were subjected to quantitative RT-PCR analysis 48 hours after treatment with the indicated concentrations of C646. The fold-change in expression of MYC is expressed relative to the level in nontreated cells. D, MYC protein expression in cells treated with C646. CBP-deficient LK2 and WT H157 cell lines were treated with C646 (0 μmol/L, C646−) or 15 μmol/L (C646+) for 48 hours, harvested, and subjected to immunoblot analysis with antibodies against MYC, acetylated histone H3K18 (H3K18ac), H3K27 (H3K27ac), and β-actin (loading control). The ratios of the levels of acetylated histone H3K18 and H3K27 levels (normalized to total H3 levels) to the corresponding levels in C646-un-treated cells are shown below. E, effect of C646 on tumor growth in vivo. CBP-deficient LK2 cells were implanted subcutaneously into BALB/c-nu/nu mice. Twenty days after implantation, the mice were randomly divided into two groups and intraperitoneally injected with C646 (25 mg/kg) or vehicle alone once a day for 14 days (arrows). Asterisks indicate significant differences in tumor volume between the C646-treated and mock-treated mice (P < 0.05; Student t test). Data, means ± SD.

CBP-deficient lung cancer cells (Fig. 5E), without any significant loss in body weight (Supplementary Fig. S5I). Thus, both in vitro and in vivo growth of CBP-deficient lung cancer cells depends on p300 HAT activity. C646 also exhibited therapeutic potential in the deadliest type of lung cancer, small cell lung cancer (SCLC), which frequently harbors CBP deficiency (ref. 31; Supplementary Fig. SSG and SSSH). Thus, CBP-deficient lung cancers are vulnerable to p300 inhibition irrespective of histologic subtypes.

We next investigated the therapeutic potential of other compounds that inhibit p300 activity. L002, multiple HAT inhibitor that targets not only p300 but also other HATs such as CBP, PCAF, and GCN5 (43), did not exert specific toxicity according to the CBP status of lung cancer cell lines (Supplementary Fig. SSI). On the other hand, lung cancer cells carrying deleterious CBP mutations were sensitive to two bromodomain inhibitors targeting p300 and CBP, SGC-CBP30 and I-CBP112 (refs. 44, 45; Supplementary Fig. SSI). These observations suggest that growth suppression of CBP-deficient lung cancer cells can be achieved by specific inhibition of p300 activity by targeting its bromodomain as well as its HAT activity.

CBP-Deficient Hematopoietic Cancer Cells Depend on p300 Function

The CBP gene is frequently mutated in hematopoietic cancers (22, 32–37). Notably, among cancers deposited in the International Cancer Genome Consortium database (https://icgc.org/), malignant lymphoma exhibits the highest rate of CBP mutation (18.2%; Supplementary Fig. S6A). Hence, we examined the effects of C646 on the growth of hematopoietic cancer cells, including malignant lymphoma, carrying CBP mutations (33–35). As observed in lung cancer cells, C646 treatment markedly reduced the proliferation of cancer cells harboring a loss-of-function mutation in the CBP domain (WSU-NHL) or deleterious mutations in the CBP gene (SU-DHL-6, VAL, Jurkat, and SU-DHL-5; Fig. 6A and Supplementary Fig. S6B).
**Figure 6.** Depletion or inhibition of p300 suppresses growth of hematopoietic cancers with loss-of-function mutations in CBP. **A,** human hematopoietic cancer cell lines used to examine sensitivity to C646. Data regarding missense and deleterious (Del) mutations were obtained from the cBioPortal and COSMIC databases. Functional impact values (neutral, low, medium, and high) of missense mutations were obtained from the MutationAssessor database. Proliferation of hematopoietic cancer cell lines was examined 5 days after treatment with or without 10 µmol/L C646. Proliferation of C646-treated cells is expressed as a percentage of proliferation in nontreated cells. Data, means ± SD. **B–D,** induction of apoptosis by C646 treatment. Jurkat (CBP-deficient) cells were treated with C646 (0, 10, or 15 µmol/L) for 6, 16, or 48 hours, and then assayed by flow cytometry to determine the cell-cycle profile (B) and the proportion of Annexin V-positive apoptotic cells (C). Data, means ± SD. Immunoblot analysis (D) was performed using antibodies against MYC, histone H3 AcK18 (H3K18ac), histone H3 AcK27 (H3K27ac), cleaved PARP, and β-actin (loading control). The ratios of the levels of acetylated histone H3K18 and H3K27 levels (normalized to total H3 levels) to the corresponding levels in C646-ununtreated cells are shown below. **E and F,** p300 depletion causes lethality of CBP-deficient hematopoietic cancer cells. Jurkat-shNT and Jurkat-shp300 cells were cultured in the presence or absence of doxycycline (Dox) before analysis. **E,** immunoblot analysis. **F,** cell proliferation assessed by cell-counting assay. Data, means ± SD. **G and H,** Induction of apoptosis by depletion of p300. Jurkat-shNT and Jurkat-shp300 cells were cultured in the presence or absence of doxycycline. After harvest, cell-cycle profiles and the proportions of Annexin V-positive apoptotic cells were assayed by flow cytometry. Data, means ± SD. **I and J,** suppression of tumor growth in vivo by p300 depletion. Jurkat-shp300 cells were implanted subcutaneously into BALB/c-nu/nu mice. When the tumors reached more than 200 mm³, mice were randomly divided into two groups and fed either a diet containing doxycycline (Dox) or a control diet (Dox−). Changes in tumor volume in both groups are shown (I). Tumor weights at the time of sacrifice in both groups (J). **K,** suppression of tumor growth in vivo by a p300-HAT inhibitor C646. CBP-deficient Jurkat cells were implanted subcutaneously into BALB/c-nu/nu mice, and the growth of xenografts was examined. Twenty days after implantation, the mice were randomly divided into two groups and intraperitoneally injected with C646 (25 mg/kg) or vehicle alone once a day for 14 days (arrows). Asterisks indicate significant differences in tumor volume between doxycycline-fed and control mice (P < 0.05; Student’s t test). Data, means ± SD.
These results strongly indicate that the growth of CBP-deficient hematopoietic cancer cells can also be suppressed by drugs that inhibit the HAT activity of p300 protein. On the other hand, CBP-deficient hematopoietic cancer cells were not specifically sensitive to the p300/CBP bromodomain inhibitors described above (Supplementary Fig. S6C). This might be due to the fact that bromodomain inhibitors have multiple targets, such as BRD4, which is therapeutically targeted in hematopoietic cancer to downregulate MYC (44).

C646 treatment led to an increase in the proportion of apoptotic cells in CBP-deficient hematopoietic cancer cells (Fig. 6B and C). Similarly, C646 treatment also led to the induction of apoptosis in other CBP-deficient, but not CBP WT, cancer cells (Supplementary Fig. S6D). C646 treatment of CBP-deficient cancer cells also reduced the levels of MYC protein, slightly decreased global histone H3 acetylation, and promoted the cleavage of PARP, a marker of apoptosis (Fig. 6D). To further examine the effect of p300 inhibition, we established CBP-deficient hematopoietic Jurkat cells in which p300 expression could be conditionally knocked down by the addition of doxycycline. Consistent with the results of C646 treatment, shRNA-mediated p300 depletion led to a reduction in cell growth, concomitant with an increase in the proportion of apoptotic cells (Fig. 6E–H and Supplementary Fig. S6E). In addition, as in the case of C646 treatment, the levels of MYC protein, acetylated histone H3, and PARP cleavage were also increased by doxycycline-induced p300 depletion (Fig. 6E). These findings suggest that p300 inhibition represents a viable therapeutic strategy against CBP-deficient hematopoietic cancers.

We next investigated the in vivo therapeutic potential of p300 inhibition against CBP-deficient hematopoietic cancers using a mouse xenograft model. To this end, we injected Jurkat cells carrying conditionally expressed nontargeting (shNT) or p300-targeting (shp300) shRNAs into the flanks of immune-deficient mice. When mice with engrafted tumors derived from CBP-deficient Jurkat cells were fed doxycycline to induce p300 depletion, the growth of Jurkat-shp300 xenografts, but not Jurkat-shNT xenografts, was significantly impaired (Fig. 6I and J and Supplementary Fig. S6F). Finally, we examined the therapeutic potential of C646 in a mouse xenograft model. C646 treatment significantly decreased the growth of CBP-deficient Jurkat cancer cells (Fig. 6K). Thus, both in vitro and in vivo growth of CBP-deficient hematopoietic cancer cells depends on p300-HAT activity, further confirming the feasibility of p300 targeting as a method for treatment of CBP-deficient cancers.

DISCUSSION

Here, we identified p300 HAT as a promising candidate for a paralog targeting strategy for treating cancers that harbor deleterious aberrations of the chromatin modifier CBP. p300 inhibition in CBP-deficient cancer cells caused downregulation of MYC and specifically led to the death of CBP-deficient cancer cells; thus, the targeting of p300 holds great promise for the treatment of CBP-deficient cancers (Fig. 7). The frequency and prevalence of mutations that inactivate chromatin modifiers in a wide range of human cancers indicate that such mutations are driver alterations that cause loss of tumor-suppressive functions (14). The promise of paralog targeting in precision cancer medicine has been further validated by the addition of the strategy described here to the previously established repertoire, e.g., the use of SMARCA2/BRM-ATPase inhibition against SMARCA4/BRG1-deficient cancers and ARID1B inhibition against ARID1A-deficient cancers.

Figure 7. Targeting p300 in CBP-deficient cancers causes synthetic lethality via apoptotic cell death due to impairment of MYC expression.
Targeting p300 Addiction in CBP-Deficient Cancers

(19–23). Thus, the paralog targeting strategy should be applicable to a considerable fraction of human cancers. We revealed the synthetic-lethal relationship between CBP and p300 using human cancer cells whose CBP or p300 genes were artificially knocked out by genome editing. Cancer cells with inactivating CBP mutations (i.e., deleterious mutations with high functional impact) and WT p300 were evidently more susceptible to p300 suppression than cells with WT or minimally compromised CBP, indicating that cancer cells with CBP deficiency have become addicted to p300 activity. On the other hand, siRNA-mediated transient double depletion of CBP and p300 in cells proficient for both genes did not cause evident lethality. This result is consistent with a previous finding that conditional knockout of both the Cbp and p300 genes in mice leads to cell-cycle arrest, but not apoptosis (24). The profound addiction to p300 inhibition exhibited by CBP-deficient cancer cells is critical for translation of p300-inhibitory therapy to the clinic.

Our findings indicate that MYC plays a key role in determining the survival of CBP-deficient cancer cells in the context of p300 depletion. Expression of MYC in CBP-deficient cancer cells was downregulated following either RNA interference– or HAT inhibitor–mediated inhibition of p300. This finding is reasonable in light of several previous studies showing that MYC upregulates the expression of cell growth–related and antiapoptotic factors that interact with CBP and p300 (46–49). Our genome-wide expression profiling data support these findings: expression of genes involved in cell growth and apoptosis-related pathways was significantly altered by p300 depletion specifically in CBP-deficient cancer cells. Furthermore, we showed that CBP and p300 HAT activity contributes to positive transcriptional regulation of the MYC gene by redundantly localizing upstream of the MYC gene TSS and acetylating histones H3K18 and H3K27, thereby promoting transcription initiation. Thus, p300 depletion–mediated transcriptional suppression of MYC, caused by impairment of histone acetylation at the MYC TSS, contributes to the vulnerability of CBP-deficient cancer cells. On the other hand, expression of a number of genes involved in cell growth and apoptosis pathways, including E2F1, E2F2, and its transcriptional targets CCND1 and CDC45, was suppressed upon depletion of p300 in CBP-deficient cancer cells. The transcriptional suppression of these genes was rescued by exogenous MYC expression, and the impaired growth was rescued only by MYC, suggesting that MYC plays key roles in the vulnerability of CBP-deficient cancer cells to p300 addiction.

CBP and p300 colocalize with acetylated H3K27 in promoter regions, whereas BRD4 also colocalizes with acetylated H3K27 on the MYC gene promoter, suggesting that these proteins could contribute to the upregulation of MYC gene expression (50–52). In fact, cancer cells with MYC-activating genomic aberrations are sensitive to BRD4 inhibitors, which cause downregulation of MYC expression and induce G1 arrest and/or apoptosis (53–55). These consequences are quite similar to those induced by p300 suppression in CBP-deficient cancer cells. On that basis, we conclude that cancer cells driven by deleterious CBP aberrations have acquired a specific cellular context that is highly dependent on MYC expression for survival, as in the case of MYC-driven tumors. Moreover, p300 depletion in CBP-deficient cancer cells did not significantly affect the accumulation of BRD4 at the MYC promoter (Supplementary Fig. S7), and CBP-deficient Jurkat cells are not susceptible to a BRD inhibitor (50, 54); therefore, the vulnerability of CBP-deficient cancer cells to p300 inhibition is likely to be achieved in a BRD4-independent manner.

Our results revealed the therapeutic utility of the existing p300 HAT inhibitor C646 (42) for personalized cancer therapy based on specific genetic alterations. CBP-mutant lung and hematopoietic cancer cells were more sensitive than WT cells to C646, reflecting a cellular context vulnerable to p300 inhibition. In particular, cancer cells carrying gross deletions, protein-truncating (nonsense and frameshift) mutations, or missense mutations in the HAT domain, as well as other predicted loss-of-function mutations, exhibited higher sensitivity to C646 than those harboring low- or medium-impact missense mutations in CBP. Therefore, patients with cancers carrying such deleterious CBP aberrations should benefit from therapy with p300 HAT inhibitors. CBP mutations in cancer cells are often heterozygous (leaving one CBP allele intact), indicating that haploinsufficiency of CBP or dominant-negative activities of mutant CBP can drive tumorigenesis (6, 30). In fact, cancer cell lines such as H1703, H520, Jurkat, VAL, and WSU-NHL, which were sensitive to p300 inhibition, harbor heterozygous CBP mutations. CBP protein was recruited to the MYC promoter region in cancer cells harboring heterozygous CBP mutations; however, it remains unclear whether mutant CBP protein was also recruited, because the WT and mutant CBP proteins could not be distinguished using the available antibodies. Moreover, SU-DHL-5 cells, which have a hemizygous focal CBP deletion that leaves the remaining allele intact, were also sensitive to C646. Thus, not only cancer cells with CBP-deficient mutations, but also those with hemizygous CBP deletion, might be vulnerable to p300-inhibitory therapy. Because C646 is an experimental drug that is not intended for use as a medication, more active p300-HAT inhibitors are required for use in the cancer clinic. We are currently attempting to develop such compounds. In addition, our results indicated that bromodomain inhibitors with high specificity for p300 could also be clinically useful.

In summary, we developed a novel therapeutic strategy for targeting CBP-deficient cancers and clearly demonstrated the therapeutic potential of this strategy in lung and hematopoietic cancer cells. Our results revealed that CBP was responsible for vulnerability to p300 depletion via a synthetic-lethal interaction between the two genes. According to the data in The Cancer Genome Atlas, many cancers harboring CBP mutations carry an intact p300 gene, indicating that the synthetic-lethal relationship between the CBP and p300 genes is conserved in other types of cancer (Supplementary Table S4; P value for mutually exclusivity = 1.0 × 10−7; Fisher exact test). However, it is possible that mutations in other genes involved in chromatin regulation might also affect this vulnerability, because cancer genomes frequently contain aberrations in a variety of those genes. In fact, a recent study newly identified KAT5B as a gene that is frequently deficient in lung cancer (38). Therefore, to obtain a complete picture of vulnerability to depletion of p300 and other HATs, further studies are warranted. A subset of diffuse large B-cell lymphomas (DLBCL)
harbor activating mutations in the EZH2 gene, which encodes a histone methyltransferase (56, 57), and such cells are sensitive to EZH2 inhibitors (57–59). However, EZH2 mutations are mutually exclusive with CBP mutations. Similarly, CBP mutations in lung squamous cell carcinomas and stomach adenocarcinomas are mutually exclusive with amplification of druggable genes such as FGFR1 and ERBB2 (Supplementary Fig. S8). Thus, p300-inhibitory therapy holds promise as a “synthetic lethal–based therapy” for treating a variety of human cancers with deleterious CBP aberrations. Notably, however, a small subset (<1%) of tumors have mutations in both the CBP and p300 genes, as shown in Supplementary Fig. S8, and such coinactivation, probably accomplished by functional complementation by other HATs, may represent a mechanism of resistance to synthetic-lethal therapy, as discussed in the context of BRM/SMARCA2 deletion therapy against cancers with BRG1/SMARCA4 deficiency (19, 60). This issue should also be investigated further.

**METHODS**

**Materials**

The lung cancer cell lines H322, H157, H1703, H520, H1299, H2009, and H2087 were provided from the establishments, Drs. John D. Minna and Adi F. Gazdar (The University of Texas Southwestern Medical Center, Dallas, TX) in 2001, and the KM12C colon cancer cell line was provided from the establishment, Dr. Isaiah J. Fidler (The University of Texas MD Anderson Cancer Center, Houston, TX) in 2000. The LK2 lung and LoVo colon cancer cell lines were purchased from the Japanese Collection of Research BioResources (JCRB) Cell Bank in April 2001 and August 1989, respectively. A lung cell line, SQ5, and two esophagus cancer cell lines, TE8 and TE10, were purchased from American Type Culture Collection (ATCC) (Rockville, MD). A brain cell line, HEK293T (July 2013); fetal lung fibroblast cell lines HFL1 and MRC5, and two esophagus cancer cell lines, TE8 and TE10, were purchased from American Type Culture Collection (ATCC) (Rockville, MD). A brain cell line, HEK293T (July 2013); fetal lung fibroblast cell lines HFL1 and MRC5 (June 2011); and a hTERT-immortalized retinal pigment epithelial cell line, hTERT-RPE-1 (June 2013), were purchased from the ATCC. Lymphoma cell lines RC-K8, SU-DHL-5, SU-DHL-6, U-2932, VAL, and WSU-NHL were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in June 2011. We authenticated the AS49, H1703, H2009, H2087, H322, LK2, H157, H520, H661, H1299, and SQ5 cell lines by verifying known cancer-related gene alterations by Sanger sequencing in 2009 (61). We authenticated the A549, H1703, H2009, H2087, H322, LK2, H157, H520, H661, H1299, and HCT116 cell lines in 2013 (19) and the KM12C, Jurkat, SU-DHL-5, VAL, and WSU-NHL cell lines in 2013–2014 by verifying alterations of multiple cancer-related genes by target sequencing using the Ion PGM system. Other cell lines have not been further authenticated by other HATs, may represent a mechanism of resistance to synthetic-lethal therapy, as discussed in the context of BRM/SMARCA2 deletion therapy against cancers with BRG1/SMARCA4 deficiency (19, 60). This issue should also be investigated further.

**Cell Proliferation Assay**

Cell proliferation was examined by measuring cellular ATP levels using the CellTiter-Glo Luminescent Cell Viability Assay. To measure cell proliferation after siRNA-mediated knockdown, cell lines were transfected with siRNAs (20–50 nmol/L) using Lipofectamine RNAiMAX reagent. After 48 hours, the cells were trypsinized, counted, and reseeded at a specified density in 96-well plates. To measure cell proliferation after treatment with C646, cells were trypsinized, counted, and reseeded at a specified density in 96-well plates, and then exposed to the indicated concentrations of C646, SGC-CBP30, or I-CBP112. Cell viability was measured using the CellTiter-Glo Luminescent Cell Viability Assay; luminescence was measured in an Envision Multi-label plate reader (PerkinElmer).

** Colony Formation Assay**

The effect of siRNA-KD on cancer cell survival was evaluated in colony formation assays. Briefly, cancer cell lines were transfected with siRNAs (20–50 nmol/L) using Lipofectamine RNAiMAX. After 48 hours, the cells were trypsinized, counted, and reseeded at a specified density in 6-well dishes, and cultured for a further 10 to 14 days to allow colony formation. To measure cell survival after treatment with C646, SGC-CBP30, I-CBP112, and L002, cells were trypsinized, counted, and reseeded at a specified density in 6-well plates, and then exposed to the indicated concentrations of the drugs for 10 to 14 days. The cells were then fixed for 5 minutes in 50% (v/w) ethanol and 0.01% (w/v) crystal violet.

** Genome-Wide Gene Expression Profiling**

BMP and p300 WT H1299 cancer cells, BMP-KO H1299 cells, and p300-KO H1299 cells were transfected with siRNAs (sINT, sip300 D1, sICBP D2) using the Lipofectamine RNAiMAX reagent. After 48 hours, total RNA was extracted using the Qiagen RNeasy kit. The integrity of the extracted RNA was confirmed by NanoDrop spectrophotometry (NanoDrop Technologies). Total RNA was reverse-transcribed using the Agilent Low Input Quick Amp Labeling Kit (Agilent Technologies). cDNA was hybridized in duplicate to Agilent microarrays (SurePrint G3 Human Gene Expression 8 × 60K Ver.1, 0.01% (v/w) crystal violet.

**Materials**

The lung cancer cell lines H322, H157, H1703, H520, H1299, H2009, and H2087 were provided from the establishments, Drs. John D. Minna and Adi F. Gazdar (The University of Texas Southwestern Medical Center, Dallas, TX) in 2001, and the KM12C colon cancer cell line was provided from the establishment, Dr. Isaiah J. Fidler (The University of Texas MD Anderson Cancer Center, Houston, TX) in 2000. The LK2 lung and LoVo colon cancer cell lines were purchased from the Japanese Collection of Research BioResources (JCRB) Cell Bank in April 2001 and August 1989, respectively. A lung cell line, SQ5, and two esophagus cancer cell lines, TE8 and TE10, were purchased from American Type Culture Collection (ATCC) (Rockville, MD). A brain cell line, HEK293T (July 2013); fetal lung fibroblast cell lines HFL1 and MRC5, and two esophagus cancer cell lines, TE8 and TE10, were purchased from American Type Culture Collection (ATCC) (Rockville, MD). A brain cell line, HEK293T (July 2013); fetal lung fibroblast cell lines HFL1 and MRC5 (June 2011); and a hTERT-immortalized retinal pigment epithelial cell line, hTERT-RPE-1 (June 2013), were purchased from the ATCC. Lymphoma cell lines RC-K8, SU-DHL-5, SU-DHL-6, U-2932, VAL, and WSU-NHL were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in June 2011. We authenticated the AS49, H1703, H2009, H2087, H322, LK2, H157, H520, H661, H1299, and SQ5 cell lines by verifying known cancer-related gene alterations by Sanger sequencing in 2009 (61). We authenticated the AS49, H1703, H2009, H2087, H322, LK2, H157, H520, H661, H1299, and HCT116 cell lines in 2013 (19) and the KM12C, Jurkat, SU-DHL-5, VAL, and WSU-NHL cell lines in 2013–2014 by verifying alterations of multiple cancer-related genes by target sequencing using the Ion PGM system. Other cell lines have not been further authenticated by us. We used cell lines for assays within 3 months after resuscitation. Supplementary Table S5 shows the status of CBP, p300, and other genes involved in chromatin remodeling/modification in these cells. Cell lines were cultured in RPMI-1640 (Wako) or DMEM (Wako) supplemented with 10% FBS (GIBCO; Life Technologies), 2 mmol/l glutamine, 100 u/mL penicillin, and 100 μg/mL streptomycin (Wako). H1299 CBP-KO cells (2G2) bearing a 26-bp deletion in exon 1 of p300 were constructed using the GeneArt CRISPR Nuclease Vector with the CD4 Enrichment Kit (Life Technologies). siRNA transfection was performed using the Lipofectamine RNAiMAX reagent (Invitrogen). The siRNAs used in this study are listed in Supplementary Table S6. Antibodies used in this study are listed in Supplementary Table S7.

** siRNA Library Screen**

Noncancerous cells (HFL1 and MRC5), CBP WT cancer cells (A549), and CBP-deficient cancer cells (LK2) were used in screening assay. Lack of mutations in the CBP, p300, SMARCA4, ARID1A, and ARID2 genes in the HFL1, MRC5, and AS49 cell lines, and the presence of homozygous CBP deletion and absence of mutations in the other genes in the LK2 cell line, were verified by targeted genome capture and massively parallel sequencing using a MiSeq sequencer and a 90-gene targeted panel, the NCC oncopanel (Cat No. 931196; Agilent). Cells seeded in 96-well plates were transfected with 50 nmol/L siRNAs targeting genes related to chromatin regulation (pools of three different siRNAs targeted each gene; Ambion) using the Lipofectamine RNAiMAX reagent (Invitrogen). Cell viability was assessed after 5 days using the CellTiter-Glo Luminescent Cell Viability Assay (Promega). The luminescence reading for each well on duplicate plates was expressed relative to the luminescence value of wells transfected with nontargeting siRNA. A result was considered a potential synthetic-lethal hit if survival under the simultaneous knockdown condition was less than 35% in CBP-deficient cells and more than 65% in noncancerous or CBP WT cells.
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G4851: 42,405 probes) using a Gene Expression Hybridization Kit (Agilent Technologies) for 16 hours at 65°C. After the arrays were washed using the Gene Expression Wash Pack (Agilent Technologies), the data were extracted using an Agilent scanner. The arrays were first analyzed using the Feature Extraction software (Agilent Technologies). A quantitative signal and qualitative detection call were generated for each sample and transcript.

Data files were subsequently analyzed utilizing GeneSpring GX12.6 (Agilent Technologies). The raw expression data of 42,545 probe sets on SurePrint G3 Human Gene Expression arrays were processed and log2-transformed. Probe sets with average log2 expression levels less than 4 were considered quantifiable, and therefore were subjected to the analysis for the remaining 42,485 probe sets. Expression data for each sample were normalized to their median expression values in the siNT-treated condition. Genes were grouped by fold changes as described in Fig. 2A. All raw microarray data files have been deposited in the Gene Expression Omnibus (GSE73682).

Quantification of mRNA and Protein

Cells were transfected with siRNAs and incubated for 48 hours. mRNA was extracted, and cDNA was synthesized using the Super-Prep Cell Lysis and RT Kit for qPCR (Toyobo). Aliquots of cDNA were subjected to quantitative PCR using the SuperPrep/THUNDERBIRD Probe qPCR Set (Toyobo) and TaqMan Gene Expression Assays (Life Technologies); the gene-specific primer/probe sets are listed in Supplementary Table S8. PCR was performed in an ABI ViiA7 Real-Time System (Life Technologies); the gene-specific primer/probe sets are listed in Supplementary Table S9 and the SuperPrep/THUNDERBIRD SYBR Green Master Mix (Life Technologies). The raw signal and qualitative detection call were generated using the Multi Gauge software V3.1 (Fujifilm).

Generation of shRNA and cDNA Expression Lentiviruses and Virus-Infected Cells

The shRNA-expressing lentiviral vector pTRIPZ (shNT, OHS5832; shp300, RHS4696-101353787; Open Biosystems) and the Lentiviral Packaging System (Open Biosystems) were used for tet-on-based shRNA expression. shRNA-expressing lentiviral vectors (shLuc, pLKO.1-shLuciferase; shp300-1/2, pLKO.1-shp300-1/2; Active Motif), or RNA polymerase II (Active Motif). Purified DNA was subjected to quantitative PCR using primer pairs listed in Supplementary Table S9 and the SuperPrep/THUNDERBIRD SYBR Green Master Mix (Life Technologies). The raw signal and qualitative detection call were generated using the Multi Gauge software V3.1 (Fujifilm).

ChIP Assay

ChIP assays were performed as previously described (1). Briefly, 1 × 106 cells were harvested 48 hours after transfection with siRNA or treatment with C646, and then treated with 1× formaldehyde for 10 minutes at room temperature to cross-link proteins to DNA. Glycine (0.125 mol/L) was added to stop the cross-linking process. ChIP assays were performed using the ChIP-IT Express Enzymatic Kit (Active Motif) and normal IgG (Cell Signaling Technology), or antibodies against CBP (Santa Cruz Biotechnology), p300 (Santa Cruz Biotechnology), histone H3K18 (Active Motif), histone H3K27 (Active Motif), or RNA polymerase II (Active Motif). Purified DNA was subjected to quantitative PCR using primer pairs listed in Supplementary Table S9 and the SuperPrep/THUNDERBIRD SYBR Green Master Mix (Life Technologies). The raw signal and qualitative detection call were generated using the Multi Gauge software V3.1 (Fujifilm).

Mouse Xenograft Model

Cells were counted and resuspended in a 1:1 mixture of culture medium and Matrigel (BD Biosciences) on ice. The cells were then injected (0.5–2 × 106 cells/mouse for H1299 and LK2; 0.5–1 × 106 cells/mouse for Jurkat cells) subcutaneously into the flank or thoracic region of nude mice (Clea, Japan) using a protocol approved by the Ethical Committee on Animal Experiments at the National Cancer Center. In the subcutaneous model, once the tumors were palpable (about 14–20 days after implantation), the mice were randomly divided into two groups. For the doxycycline treatment method, mice were fed either a diet containing doxycycline (400 ppm) or a control diet. In the drug treatment method, mice were injected intraperitoneally with C646 (25 mg/kg; Tocris) once a day for 14 days. Tumor growth was measured every 3 to 4 days using calipers. In the orthotopic model (63), LK2 shp300 cells were
treated with or without doxycycline for 4 days, and then subsequently injected into the thoracic cavity followed by feeding diet containing doxycycline (400 ppm) or control diet. Tumor growth was measured 24 days after injection. The volume of the implanted tumors was calculated using the formula $V = L \times W^2/2$, where $V$ is volume (mm$^3$), $L$ is the largest diameter (mm), and $W$ is the smallest diameter (mm). At the end of the experiment, the mice were sacrificed in accordance with standard protocols.

**Statistical Analysis**

All experiments were performed in triplicate, and data are expressed as means ± SD. Data from mouse xenograft models are expressed as means ± SE. Differences between drug-treated and untreated cells were evaluated using the Student $t$ test. Statistically significant differences are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: H. Ogiwara

Development of methodology: H. Ogiwara

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Ogiwara, M. Sasaki, T. Oike, S. Higuchi, Y. Tominaga

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Ogiwara

Writing, review, and/or revision of the manuscript: H. Ogiwara, T. Kohno

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Ogiwara, T. Mitachi

Study supervision: H. Ogiwara, T. Kohno

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Targeting p300 Addiction in CBP-Deficient Cancers

Targeting p300 Addiction in CBP-Deficient Cancers Causes Synthetic Lethality by Apoptotic Cell Death due to Abrogation of MYC Expression

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