

IN THE SPOTLIGHT

Lifting Up the HAT: Synthetic Lethal Screening Reveals a Novel Vulnerability at the CBP–p300 Axis

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Summary: Cancer genotype-specific synthetic lethal vulnerabilities represent promising therapeutic targets. In this issue of *Cancer Discovery*, Ogiwara and colleagues uncover a synthetic lethal relationship between two histone acetyl transferase paralogs, CBP and p300, highlighting that cancer cells deficient in CBP are uniquely sensitized to genetic and chemical inhibition of p300. *Cancer Discov*; 6(4); 350–2. ©2016 AACR.

See related article by Ogiwara et al., p. 430 (9).

Synthetic lethal screening efforts have recently unveiled a host of new cancer genotype-specific therapeutic targets. DNA-sequencing studies in human cancer and cancer cell lines coupled with the emergence of high-throughput screening technologies have greatly enabled the discovery of disease-relevant synthetic lethal relationships. The dependencies on chromatin regulators of cancers driven by mutations in genes encoding epigenetic factors provide new opportunities to target the delicate balance governing epigenetic maintenance (1). New targets identified in these studies exploit inherent differences between cancer cells and normal cells; this is particularly advantageous in cancers driven by loss-of-function (LOF) mutations rather than mutated oncogenes with increased activity.

Synthetic lethal relationships among chromatin regulators can occur between (i) members of the same chromatin regulatory complex, often paralogs; (ii) members of different complexes, often with opposing functions on chromatin; or (iii) downstream pathways that are preferentially activated upon loss or mutation to specific genes encoding a chromatin regulator. The first relationship highlights the nonredundant, yet possibly compensatory, roles of paralogous subunits of chromatin-regulatory complexes, such as SMARCA4 (BRG1)/SMARCA2 (BRM) and ARID1A/1B of the mammalian SWI/SNF (BAF) complex (2–4). Oike and colleagues originally reported the synthetic lethality relationship between the BRG1/BRM paralogs before genome-wide shRNA and CRISPR screens were used by other groups to confirm this finding (5). The second relationship, dependence on members of different chromatin regulatory complexes, reflects the inherent balance conferred by different chromatin regulators toward maintaining epigenetic control. For example, BAF complexes (trithorax family) oppose

PRC2 (PcG family) complexes genome-wide, and hence cancers with specific BAF complex perturbations, such as SMARCB1 loss and ARID1A loss, are uniquely sensitive to inhibition of the PRC2 catalytic subunit EZH2 (6, 7). Finally, it has become clear that cancers with specific mutations in genes encoding chromatin regulators become locked in a state of oncogenic addiction, driving downstream pathways, and hence are uniquely dependent upon downstream targets that play critical roles in maintaining cell proliferation. All three types of synthetic lethal relationships have suggested new therapeutic approaches while also raising challenges in achieving the sufficient therapeutic windows requisite for translation into the clinic.

In this issue of *Cancer Discovery*, Ogiwara and colleagues uncover a novel synthetic lethal relationship between the histone acetyl transferases CBP/CREBBP and p300/EP300, two highly similar KAT3 family paralogs that share the function of placing acetyl marks at lysines K18 and K27 of histone H3 (8, 9). CBP and p300 are also transcriptional coactivators for various DNA-binding transcription factors. LOF mutations in *CBP* are present in 10% to 15% of non-small cell lung cancers, as well as several other tumor types, such as lymphoma (8), leukemia, and bladder carcinoma (Fig. 1A). In addition to gross deletions or protein-truncating LOF mutations, missense mutations appear to cluster in the histone acetyl transferase (HAT) domain, pointing to the critical function of the catalytic domain and the placement of acetyl marks on chromatin to achieve active gene expression. In addition to the frequency of *CBP* mutations in cancer, heterozygous mutations in *CBP* are responsible for a congenital neurodevelopmental disorder known as Rubinstein–Taybi syndrome, characterized by mental growth retardation and tumor predisposition (10).

The authors used functional synthetic lethal screening to discover that CBP-deficient cancer cell lines and CBP knockout cells are uniquely susceptible to p300 ablation, which results in G₁–S cell-cycle arrest and apoptosis (Fig. 1B). Genetic or chemical inhibition of p300 results in decreased *MYC* expression via loss of H3K27 and H3K18 acetylation over its promoter. The authors determined that *MYC* is a particularly important factor in this synthetic lethal relationship, as its exogenous expression is sufficient to rescue this synthetic lethality in CBP-deficient cells. Impressively, even

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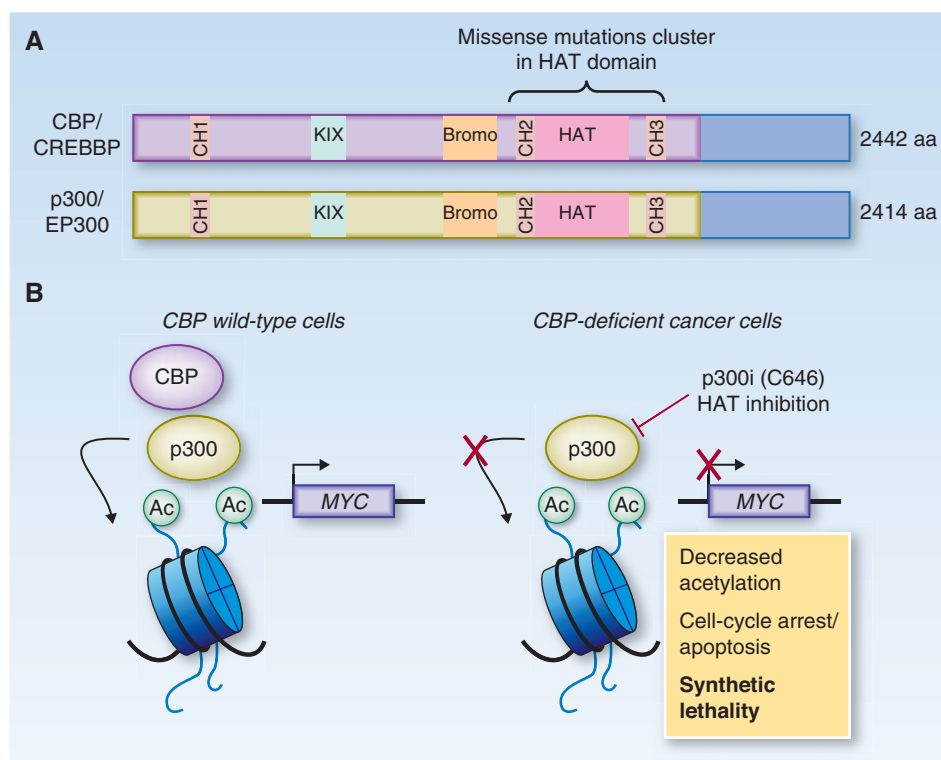


Figure 1. Targeting the CBP-p300 synthetic lethal axis. **A**, CBP (CREBBP) and p300 (EP300) protein schematics reflect highly similar structure. Cancer-associated missense mutations are clustered in the HAT domain. **B**, normal cells contain wild-type copies of both HAT paralogs, CBP and p300, which acetylate lysine tails on histones, serve as important coactivators for transcription factors, and maintain gene expression programs (such as MYC expression). In CBP-deficient cells, p300 is the only HAT paralog remaining. p300 inhibition results in decreased acetylation and inhibition of prosurvival genes such as MYC, leading to cell-cycle arrest and apoptosis.

cell lines bearing heterozygous mutations exhibited gained sensitivity to p300 inhibition, confirming that, as with most chromatin regulators, haploinsufficiency can drive tumorigenesis.

Although several other recently identified synthetic lethal vulnerabilities do not currently have targeted small-molecule therapeutics available [such as the mSWI/SNF (BAF) complex subunits SMARCA2 (in SMARCA4-deficient cancers; ref. 2); and ARID1B (in ARID1A-deficient cancers; ref. 4)], several p300 inhibitors have been generated, including C646, used in this study. The availability of p300 modulators makes this newly identified synthetic lethal relationship particularly attractive for medicinal chemistry efforts to develop leads for potential clinical studies. The results shown in this article also suggest that bromodomain inhibitors with high specificity for p300 bromodomain may exhibit selective toxicity in CBP-deficient cancers. Inspired by these data, future screening approaches will also likely continue to reveal whether deleterious mutations in other HATs, such as *KAT6B* and others, will result in similar dependencies on p300, potentially expanding the therapeutic relevance of this study.

Synthetic lethal relationships between paralogs with highly conserved sequence and function can either indicate that the remaining paralog compensates for the lost paralog and provides overlapping, redundant function as the paralog deleted, or that the balance between two highly similar but

nonredundant paralogs is shifted, leading to oncogene addition on the remaining intact member. A precise determination of the mechanisms underpinning synthetic lethal relationships is lacking in virtually all such cases and will be required to gain a complete understanding of the context-specific nature of paralog functions as well as, ultimately, selection of optimized patient populations for clinical trials. Given that *CBP* mutations (and most other mutations in chromatin-regulatory genes) often occur in the context of additional mutations in human cancers, further studies will be required to determine the specific genes that are capable of negating the CBP-p300 synthetic lethal relationship, such as *MYC* expression in this study, as these genes may represent pathways to drug resistance. The fact that this synthetic lethal vulnerability can be rescued by exogenous expression of *MYC* provides a cautionary note that amplification of prosurvival oncogenes is likely to represent mechanisms of resistance in these tumors. *MYC* upregulation in particular is one that can be achieved through multiple pathways, and hence this compensatory event in particular could emerge as a resistance mechanism. Of note, as a small subset of tumors harbor mutations in both *CBP* and *EP300*, the compensatory mechanisms and novel dependencies in this dual paralog loss setting will require further exploration, as is the case currently with other synthetic lethal pairs such as *BRG1/BRM*, which are co-silenced in specific tumor types (11).

Questions remain as to whether synthetic lethal targeting of chromatin regulators will provide a viable approach to cancer therapy, including their ability to exhibit low toxicity profiles, given that many of these targets are expressed and active in normal cells. To date, only a small number of synthetic lethal targets have progressed into clinical trials; however, these challenges are most often due to a lack of potent, specific inhibitors. In this study, p300 inhibition was significantly more toxic in CBP-deficient cells than in cells containing wild-type copies of both CBP and p300; however, in an organism treated over a long time period, ablation of a nonredundant paralog may induce cell- or tissue-specific toxicity. However, insight into these challenges can be gained when assessing cancer genotype-specific vulnerabilities against large numbers of well-characterized cell lines and complete cell type-specific knockout libraries. Moreover, for some targets, clinical trials in patients are already under way and appear promising, such as those using EZH2 inhibitors in mSWI/SNF (BAF) LOF cancer settings. Resistance mechanisms in patients following prolonged treatment with epigenetic inhibitors, especially those targeting synthetic lethal relationships between paralogs, remain to be defined.

The discovery of novel synthetic lethal relationships has fueled intense interest within the cancer and epigenetics fields toward achieving a detailed mechanistic understanding of the vulnerabilities conferred by faulty chromatin regulators and identifying opportunities for therapeutic intervention. This study reveals that the CBP/p300 synthetic lethal target is particularly ripe for intervention and represents an exciting new opportunity at the intersection of chromatin regulation, cancer, and novel small-molecule therapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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REFERENCES

1. Mair B, Kubicek S, Nijman SM. Exploiting epigenetic vulnerabilities for cancer therapeutics. *Trends Pharmacol Sci* 2014;35:136–45.
2. Hoffman GR, Rahal R, Buxton F, Xiang K, McAllister G, Frias E, et al. Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. *Proc Natl Acad Sci U S A* 2014;111:3128–33.
3. Wilson BG, Helming KC, Wang X, Kim Y, Vazquez F, Jagani Z, et al. Residual complexes containing SMARCA2 (BRM) underlie the oncogenic drive of SMARCA4 (BRG1) mutation. *Mol Cell Biol* 2014;34:1136–44.
4. Helming KC, Wang X, Wilson BG, Vazquez F, Haswell JR, Manchester HE, et al. ARID1B is a specific vulnerability in ARID1A-mutant cancers. *Nat Med* 2014;20:251–4.
5. Oike T, Ogiwara H, Tominaga Y, Ito K, Ando O, Tsuta K, et al. A synthetic lethality-based strategy to treat cancers harboring a genetic deficiency in the chromatin remodeling factor BRG1. *Cancer Res* 2013;73:5508–18.
6. Bitler BG, Aird KM, Garipov A, Li H, Amatangelo M, Kossenkov AV, et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nat Med* 2015;21:231–8.
7. Wilson BG, Wang X, Shen X, McKenna ES, Lemieux ME, Cho YJ, et al. Epigenetic antagonism between polycomb and SWI/SNF complexes during oncogenic transformation. *Cancer Cell* 2010;18:316–28.
8. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* 2011;471:189–95.
9. Ogiwara H, Sasaki M, Mitachi T, Oike T, Higuchi S, Tominaga Y, et al. Targeting p300 addiction in CBP-deficient cancers causes synthetic lethality by apoptotic cell death due to abrogation of MYC expression. *Cancer Discov* 2016;6:430–45.
10. Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, et al. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 1995;376:348–51.
11. Karnezis AN, Wang Y, Ramos P, Hendricks WP, Oliva E, D'Angelo E, et al. Dual loss of the SWI/SNF complex ATPases SMARCA4/BRG1 and SMARCA2/BRM is highly sensitive and specific for small cell carcinoma of the ovary, hypercalcaemic type. *J Pathol* 2016;238:389–400.

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