SMARCA4/2 loss inhibits chemotherapy-induced apoptosis by restricting IP3R3-mediated Ca\(^{2+}\) flux to mitochondria

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Inactivating mutations in SMARCA4 and concurrent epigenetic silencing of SMARCA2 characterize subsets of ovarian and lung cancers. Concomitant loss of these key subunits of SWI/SNF chromatin remodeling complexes in both cancers is associated with chemotherapy resistance and poor prognosis. Here, we discover that SMARCA4/2 loss inhibits chemotherapy-induced apoptosis through disrupting intracellular organelle calcium ion (Ca\(^{2+}\)) release in these cancers. By restricting chromatin accessibility to ITPR3, encoding Ca\(^{2+}\) channel IP3R3, SMARCA4/2 deficiency causes reduced IP3R3 expression leading to impaired Ca\(^{2+}\) transfer from the endoplasmic reticulum to mitochondria required for apoptosis induction. Reactivation of SMARCA2 by a histone deacetylase inhibitor rescues IP3R3 expression and enhances cisplatin response in SMARCA4/2-deficient cancer cells both in vitro and in vivo. Our findings elucidate the contribution of SMARCA4/2 to Ca\(^{2+}\)-dependent apoptosis induction, which may be exploited to enhance chemotherapy response in SMARCA4/2-deficient cancers.
The SWI/SNF family of ATP-dependent chromatin remodeling complexes control gene expression by regulating chromatin organization1–2. They also directly participate in DNA replication, repair, and recombination through modifying chromatin or recruiting relevant proteins3. Cancer genome-sequencing efforts have revealed mutations in SWI/SNF subunits in more than 20% of all human cancers, highlighting their critical roles in tumorigenesis4. However, identifying the driver mechanisms of SWI/SNF loss in promoting cancer remains a challenge.

SMARCA4 (BRG1) and SMARCA2 (BRM) are the two mutually exclusive ATPase subunits of SWI/SNF. SMARCA4 is inactivated by mutations or other mechanisms in ~10% of non-small cell lung cancer (NSCLC)5–9. Furthermore, concomitant loss of SMARCA4/2 protein expression occurs in a subset of NSCLC associated with a poor prognosis6,10. In addition to NSCLC, deleterious SMARCA4/2 mutations have been found to be the sole genetic driver in ~100% of small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), a rare and aggressive ovarian cancer affecting young women11–13. SCCOHT is also characterized by concurrent loss of SMARCA4/2 protein expression, where SMARCA4 is epigenetically silenced and its reactivation strongly suppressed SCCOHT growth14,15. In contrast to other cancer types where experimental SMARCA2 inhibition is synthetic lethal with SMARCA4 loss16–20, SMARCA2 silencing may cooperate with SMARCA4 loss in SMARCA4/2-deficient SCCOHT and NSCLC for cancer development10,21. However, the underlying mechanisms are not understood.

In addition to regulating gene expression, SWI/SNF components, including SMARCA4, have also been implicated in DNA-damage repair (DDR)22–24. Thus, their inactivation may also lead to compromised DDR and genome instability which are widely recognized as driving events in cancer development25. However, SCCOHT has a simple genome and harbors few mutations or chromosomal alterations other than inactivating mutations in SMARCA415,26,27, suggesting that altered transcriptional regulation may be the predominant driver of tumorigenesis in this cancer28.

Platinum-based chemotherapies, such as cisplatin, induce DNA damage leading to cancer cell apoptosis and have been widely used in clinical practice for treating lung and ovarian cancers29,30. The involvement of SWI/SNF in DDR supports the use of these genotoxic agents for treating cancers with SMARCA4/2 deficiency, which does not often co-occur with other druggable oncogenic mutations. Indeed, previous studies have shown that experimental inhibition of SMARCA4 in SMARCA4-proficient cancer cells enhanced response to DNA damaging agents31–33. However, conventional chemotherapies are rarely effective for SCCOHT patients34 and compared to other ovarian cancer types, SCCOHT cell lines show substantial resistance to these drugs26,35. In line with this, NSCLC patients with concomitant loss of SMARCA4/2 have a poorer prognosis than others3,10 while adjuvant chemotherapy remains among primary treatment options for this cancer29. Thus, while SWI/SNF deficiencies have been widely associated to cancer progression, the mechanism by which SMARCA4/2-deficient cancer cells have adapted to resist chemotherapy is unknown.

In this study, we sought to examine the role of SMARCA4/2 in modulating chemotherapy responses in SCCOHT and NSCLC where SMARCA4/2 deficiency is frequently observed. Our results reveal a mechanism linking SMARCA4/2 loss to chemoresistance by inhibiting apoptosis induction and suggest a potential therapeutic strategy for improving treatment for SMARCA4/2-deficient cancers.

Results

SMARCA4/2 loss confers resistance to chemo-induced apoptosis in cancer cells. SCCOHT harbors few mutations or chromosomal alterations other than inactivating mutations in SMARCA4 but is typically resistant to conventional chemotherapy in patients35,34, suggesting a potential connection between SMARCA4 deficiency and chemoresistance. Since SMARCA4 is also frequently inactivated in NSCLC, we investigated the association of SMARCA4 expression with chemoresistance in this cancer type. We first analyzed the most comprehensive NSCLC microarray gene expression data set with clinical outcome from the Director’s Challenge data set of lung adenocarcinoma (LUAD, the most common NSCLC subtype) of diverse tumor staging36. For our analysis, we chose SMARCA4 as a “probe” unbiasedly identified by Kaplan–Meier (KM) plotter37,38, which is the optimal probe set for specificity, coverage, and degradation resistance without preassociation with patient outcome. We stratified the patients within each treatment group based on median of SMARCA4 expression and found that low SMARCA4 expression was significantly associated with worse survival with adjuvant therapies (chemotherapy and radiation) when compared to high SMARCA4 expression (Supplementary Fig. 1a). This was supported by similar results obtained from KM plotter analyzing multiple available LUAD data sets of diverse tumor staging using the same probe (Supplementary Fig. 1b). A similar trend in UT lung SPORE data set39 was also observed although not statistically significant (Supplementary Fig. 1c). Together, these patient outcome results suggest that SMARCA4 deficiency is associated with chemoresistance in NSCLC, similar to that seen in SCCOHT.

Because patient outcomes from the data sets described above may be influenced by other variable factors such as treatment history, we next examined the role of SWI/SNF loss in mediating chemoresistance in more controlled experimental settings using cancer cell lines. First, we investigated the correlation between chemotherapy responses and mRNA expression levels of SMARCA4/2 in a large cohort of cell lines (n = 436) across different cancer types (Supplementary Fig. 2a), by integrating publicly available drug sensitivity data from Genomics of Drug Sensitivity in Cancer (GDSC)40 and RNA sequencing (RNA-seq) data from Cancer Cell Line Encyclopedia (CCLE)41,42. We stratified these pan cancer cell lines (n = 436) based on their SMARCA4/2 expression in tertiles (Supplementary Fig. 2b) and found that SMARCA4Low/SMARCA2Low (A4H/A2H, bottom tertile for both genes) group (n = 53) has the highest half maximal inhibitory concentration (IC50) among all four groups, for common chemotherapy drugs with different mechanisms of action, including cisplatin, cyclophosphamide, topotecan, paclitaxel, etoposide, and 5FU (Fig. 1a, Supplementary Fig. 2c). Notably, IC50 difference between A4H/A2H and the SMARCA4High/SMARCA2High (A4H/A2H top tertile for both genes) group (n = 50) was statistically significant for all of these drugs. The SMARCA4Low/SMARCA2High (A4H/A2H) group (n = 24) had the second highest IC50 which was significantly higher than that of the A4H/A2H group in three of the six drugs including cisplatin. We also observed a consistent trend of higher IC50 in the SMARCA4High/SMARCA2Low (A4H/A2L) group (n = 34) compared to A4H/A2H although it was not statistically significant. Similar results were also obtained when analyzing lung cancer cell lines only (Fig. 1b, Supplementary Fig. 2d), which represented the largest cancer type (n = 103) among the CCLE panel (Supplementary Fig. 2a). Together, these observations show that reduced SMARCA4/2 expression correlates with resistance to different chemotherapies, including cisplatin, and suggest that SMARCA4 may play a dominant role in regulating drug responses in cancer cells.

To help unbiasedly assess the potential roles of SWI/SNF genes in modulating cisplatin responses, we performed a pooled CRISPR knockout screen targeting 496 epigenetic modifiers in OVCAR4, a SMARCA4/2-proficient high-grade serous ovarian ovarian...
cancer (HGSC) cell line (Fig. 1c). Upon screen completion, we analyzed the data using the MAGeCK statistical software package\textsuperscript{43,44} to search for candidate genes whose knockout may confer cisplatin resistance. Validating the screen, we identified \textit{EP300} and \textit{CARM1} among the top candidates (ranked #1 and #5, respectively; Supplementary Table 1 and Fig. 1d), whose suppression is known to confer cisplatin resistance\textsuperscript{45–47}. In keeping with our above findings in patient outcome and CCLE cell line responses to chemotherapies, \textit{SMARCA4} was also highly ranked (#11) in our screen suggesting that \textit{SMARCA4} loss confers cisplatin resistance (Fig. 1c, d and Supplementary Table 1). \textit{SMARCA2} was not significantly enriched (ranked #162), suggesting that \textit{SMARCA4} plays a dominant role in controlling cisplatin response, with \textit{SMARCA2} only compensating when \textit{SMARCA4} is lost.

To validate the above screen results, we knocked out \textit{SMARCA4} in OVCAR4 cells using CRISPR/Cas9 genome editing system and investigated their apoptotic responses known to be induced by cisplatin treatment. Compared to the parental control, \textit{SMARCA4} knockout (A4\textsuperscript{KO}) cells were more resistant to cisplatin-induced...
Fig. 1 SMARCA4/2 loss causes resistance to chemotherapeutics in ovarian and lung cancers. The half maximal inhibitory concentration (IC_{50}) of cisplatin in pan cancer (a) and lung cancer (b) cell lines with differential mRNA expression for SMARCA4 and SMARCA2 (see Supplementary Fig. 2b for stratification). A_{40}^{40}: SMARCA4(A4); A_{20}^{40}: SMARCA4(A4); SMARCA2(A2); A_{20}^{20}: SMARCA2(A2). Cell line numbers are indicated in gray below each group. One-way ANOVA Kruskal–Wallis test followed by Dunn’s test for multiple comparisons against A_{40}^{40}A_{20}^{40} group, p values (p): a A_{40}^{40}A_{20}^{40}—0.0338, A_{40}^{40}A_{20}^{40}—0.0035, b A_{40}^{40}A_{20}^{40}—0.4615, A_{40}^{40}A_{20}^{40}—0.0517, A_{40}^{40}A_{20}^{40}—0.0019. c Schematic outline of a pooled CRISPR screen with a sgRNA knockout library against epigenetic regulators to identify genes required for cisplatin response in OVCAR4 cells. d MAGEN analysis reveals that SMARCA4/2-related genes were enriched in the CRISPR knockdowns. e Genes were ranked by robust rank aggregation (RRA). Immunoblot (e), annexin V/PI+ apoptotic cell population determined by flow cytometry (f), and representative phase-contrast images (g) of OVCAR4 cells with indicated SMARCA4/2 perturbations and cisplatin treatments (e, f 48 h). Immunoblot (h), annexin V/PI+ apoptotic cell population (i), and representative phase-contrast images (j) of H1703 cells with indicated SMARCA4/2 perturbations and cisplatin treatments (h, i 72 h). e-j Control, A_{40}^{40}KO SMARCA4 knockout, shA2 shRNA targeting SMARCA2, cl. PARP cleaved PARP, cl. caspase 3 cleaved caspase 3, A4 SMARCA4, A2 SMARCA2. Scale bar, 150 μm. Mean ± SD, n = 3 independent experiments, one-way ANOVA followed by Dunnett’s test for multiple comparisons, p values (p): f all (<0.0001); i A2 (0 μM)—0.0032, A4 (0 μM)—0.0023, A2 or A4 (3 μM) < 0.0001. **p < 0.01, ***p < 0.0001.

SMARCA4/2 loss results in altered intracellular Ca^{2+} homeostasis in cancer cells. To understand how SMARCA4/2 regulate chemotherapy sensitivity and apoptosis induction, we analyzed the transcriptome regulated by SMARCA4 using SCCOHT cells, taking advantage of their simple genetic background. Gene set enrichment analysis (GSEA) of RNA-seq data generated in SCCOHT-1 and BIN-67 cells ± SMARCA4 restoration reveals top ten Gene Ontology (GO) terms regulated by SMARCA4 consistently shared by these two SCCOHT cell lines (Supplementary Fig. 5). Multiple terms associated with ion/calcium homeostasis were identified including "ion transmembrane transporter" and "calcium ion binding" (Fig. 2a, b). The established crucial role of calcium ion (Ca^{2+}) homeostasis in apoptosis induction makes these GO terms particularly interesting. Transient Ca^{2+} release from the endoplasmic reticulum (ER), the major intracellular Ca^{2+} store, into the cytosol and subsequent transfer to mitochondria is important for cellular signal transductions as well as ATP production. However, excessive ER-Ca^{2+} release leads to mitochondrial Ca^{2+} overload and cell death, which has recently been associated to the selective vulnerability of cancer cells. Together, these transcriptome analyses in SCCOHT cell lines indicate that Ca^{2+} homeostasis may be a commonly altered cellular process by SMARCA4, contributing to their roles in apoptosis regulation and cancer cell survival.

Given the crucial role of intracellular Ca^{2+} signaling in apoptosis induction, we reasoned that SMARCA4/2 may affect apoptosis by regulating intracellular Ca^{2+} flux. To validate the role of SMARCA4/2 in Ca^{2+} homeostasis and transfer to mitochondria, we measured the changes in cytosolic and mitochondrial Ca^{2+} content of SCCOHT-1 cells, ±SMARCA4 restoration, in response to histamine, an inositol trisphosphate (IP3) agonist activating ER-Ca^{2+} release via inositol trisphosphate receptor (IP3R)45. In order to monitor intracellular Ca^{2+} dynamics, we expressed genetically encoded Ca^{2+} indicators (GECI) targeted to the cytosol (R-GECO)56 or mitochondria (CEPIA-2mt)57 and monitored GECI fluorescence upon ER-Ca^{2+} release stimulation by spinning disk confocal microscopy (Supplementary Fig. 6). While histamine stimulation induced little changes in cytosolic or mitochondrial Ca^{2+} in SCCOHT-1 control cells, it strongly elevated Ca^{2+} content in both compartments in SMARCA4-restored cells (Fig. 2c–e). Consistent with this, restoration of SMARCA4 in H1703 cells also significantly increased ER-Ca^{2+} release to the cytosol and Ca^{2+} transfer to the mitochondria upon histamine stimulation, compared to control cells (Fig. 2f–h). These data indicate that SMARCA4 plays a causative role in regulating intracellular Ca^{2+} homeostasis by enabling ER-Ca^{2+} release to the cytosol and mitochondria.

The increased cytosolic and mitochondrial Ca^{2+} content observed upon SMARCA4 restoration could be due to either direct enhanced Ca^{2+} release from the ER or elevated capacity of the ER-Ca^{2+} content. To distinguish these possibilities, we measured the cytosolic Ca^{2+} changes in above isogenic cell pairs of SCCOHT-1

Elevation of annexin V (cell death marker; Supplementary Fig. 3a), cleaved PARP, and cleaved caspase 3 (apoptosis markers; Fig. 1e). They also exhibited reduced annexin V+/propidium iodide (PI)- apoptotic cell population (Fig. 1f), and had fewer morphological defects, a characteristic of the apoptotic cell (Fig. 1g) in response to cisplatin treatment. Similarly, SMARCA4 knockout also protected OVCAR4 cells against paclitaxel-induced apoptosis (Supplementary Fig. 3b). Furthermore, knockdown of SMARCA2 using two independent short hairpin RNAs (shRNAs) in these A_{40}^{40}KO cells led to increased resistance to the above-described apoptotic responses induced by cisplatin (Fig. 1e–g, Supplementary Fig. 3a). Similar results were obtained in the HEK116 ovarian endometrial cancer cell line (Supplementary Fig. 3c, d), further validating the above results in OVCAR4 cells. We also noted that high dose of cisplatin treatment in OVCAR4 control cells led to reduced SMARCA4/2 protein expression (Fig. 1e), suggesting a potential negative feedback regulation or a selection for cells expressing low SMARCA4/2. To corroborate our results, we sought to perform the reverse experiments by restoring SMARCA4 or SMARCA2 in SMARCA4/2-deficient cancer cells. SMARCA4/2 restoration in SCCOHT cells both strongly suppressed their growth16,17, which limited the experimental window to study apoptosis regulation upon subsequent cisplatin treatment. In contrast, SMARCA4/2-deficient NSCLC cells including H1703 cells can tolerate restoration of SMARCA4/2 and thus are better suited for this analysis. Ectopic expression of SMARCA4 or SMARCA2 sensitized H1703 cells to cisplatin treatment and led to strong induction of apoptosis, indicated by elevation of annexin V, cleaved PARP, and cleaved caspase 3, a marked increase of the annexin V+/PI- apoptotic cell population, acquisition of apoptotic cell morphology, and impaired growth (Fig. 1h–j, Supplementary Fig. 3e, f). Further supporting this, CRISPR/Cas9-mediated SMARCA4 knockout in SMARCA4/2-proficient H1437 NSCLC cancer cells conferred resistance to apoptosis induced by cisplatin treatment; knockdown of SMARCA2 in these A_{40}^{40}KO cells led to further increased resistance to cisplatin, indicated by reduction of cleaved PARP and cleaved caspase 3 and increased cell viability (Supplementary Fig. 3g, h).

We further examined the effect of SMARCA4 loss in response to other common chemotherapeutics using above-described isogenic cell pairs of HEC116 and H1703 that differ only in SMARCA4 status. Consistent with cisplatin results, SMARCA4 knockout in HEC116 cells suppressed elevation of cleaved PARP and cleaved caspase 3 induced by cyclophosphamide, topotecan, and paclitaxel (Supplementary Fig. 4a) and led to increased cell viability in the presence of these agents (Supplementary Fig. 4b). Conversely, SMARCA4 restoration sensitized H1703 cells to the treatment with these drugs, as indicated by elevation of apoptosis and increased cell viability (Supplementary Fig. 4c, d). Together, our data indicate that SMARCA4/2 loss inhibits chemotherapeutic-induced apoptotic responses in ovarian and lung cancer cells.
and H1703 in response to thapsigargin, an inhibitor of sarcoplasmic/ER Ca\(^{2+}\)-ATPase, which can entirely deplete ER-Ca\(^{2+}\) stores\(^5\). Interestingly, restoration of SMARCA4 does not increase maximal cytosolic ER-Ca\(^{2+}\) release induced by thapsigargin treatment in SCCOHT-1 or H1703 cells (Supplementary Fig. 7a, b), suggesting that SMARCA4 promotes Ca\(^{2+}\) release from the ER rather than an increase in ER-Ca\(^{2+}\) storage capacity. Further supporting this, SMARCA4 knockout in OVCAR4 and H1437 cells significantly decreased the induction of cytosolic and mitochondrial Ca\(^{2+}\) upon histamine treatment (Fig. 2i–n), even though SMARCA4 knockout had increased ER-Ca\(^{2+}\) stores as indicated by an increase in cytosolic Ca\(^{2+}\) in OVCAR4 cells, but not in H1437 cells, following thapsigargin stimulation (Supplementary Fig. 7c, d). Finally, to rule out the potential contribution of the mitochondrial Ca\(^{2+}\) uptake
machinery in this phenotype, we showed that protein levels of the mitochondrial calcium uniporter (MCU) and its regulators were unchanged in these cell lines, indicating that Ca++ transfer defects were not due to defective mitochondrial Ca++ import machinery (Supplementary Fig. 7e). Together, these results suggest that SMARCA4/2 regulate intracellular Ca++ homeostasis and mitochondrial Ca++ content likely by controlling Ca++ release from the ER.

SMARCA4/2 directly regulate ITPR3 expression. To dissect the detailed mechanism by which SMARCA4/2 regulate Ca+++ homeostasis, we further investigated Ca+++-related genes in ion/calcium associated GO terms identified from the above transcriptome analysis in SCCOHT cells (Fig. 2a, b). Overlapping the two datasets yielded 198 common genes affected by SMARCA4 restoration in both SCCOHT-1 and BIN-67 cells (Fig. 3a; Supplementary Table 2). To help identify direct targets of SMARCA4, we examined these 198 commonly regulated genes in a chromatin immunoprecipitation sequencing (ChIP-seq) data set profiling SMARCA4 occupancy in BIN-67 cells ± SMARCA4 restoration. This analysis revealed 69 of the 198 genes showing SMARCA4 occupancy in their loci (Fig. 3a; Supplementary Table 2). Considering that SMARCA4 and SMARCA2 may regulate the same target genes and that SMARCA4 also modulates Ca++ homeostasis in NSCLC cells (Fig. 2f–h), we then examined the regulation of these 69 genes in an independent RNA-seq data set of BIN-67 cells ± SMARCA4/2 restoration and a microarray data set of NSCLC cell line H1299 ± SMARCA4 restoration. Notably, all of the 69 SMARCA4-affected genes were also regulated by SMARCA2 in BIN-67 cells, indicating that SMARCA4/2 may have redundant function in controlling Ca++ homeostasis (Fig. 3b). In keeping with the fact that lung cancer cells have more complex genetic landscapes than SCCOHT,15,62, only four genes, namely ITPR3, MATN2, EHD4, and ATP2B4, were consistently upregulated by SMARCA4 in both cancer types (Fig. 3b).

Among these four common genes, ITPR3 encodes inositol 1,4,5-trisphosphate receptor type 3 (IP3R3), one of the IP3R family members that forms Ca+++ channels on the ER and plays critical roles in intracellular Ca++ homeostasis and cell apoptosis. IP3R3 localizes at the mitochondrial-associated membranes, a signaling platform allowing the generation of microdomains of high Ca+++ concentration required for efficient mitochondrial Ca++ uptake, and preferentially transmits apoptotic Ca++ signals into mitochondria over other IP3Rs. Tumor suppressors such as PTEN, BAP1, and PML have been shown to induce apoptosis in cancer cell by promoting IP3R3-mediated Ca+++ flux from the ER to mitochondria. Thus, we hypothesized that SMARCA4/2 may promote Ca+++ flux to the mitochondria and apoptosis induction by directly regulating ITPR3 gene expression. Corroborating our transcriptome data above (Fig. 3b), ectopic expression of SMARCA4 or SMARCA2 in both SCCOHT (BIN-67, SCCOHT-1) and NSCLC (H1299, H1703) cells resulted in elevated mRNA and protein expression of IP3R3 (Fig. 3c, d). Conversely, SMARCA4 knockout in OVCAR4, HEC116, and H1437 cells suppressed IP3R3 expression which was further downregulated upon subsequent SMARCA2 knockout (Supplementary Fig. 8). These data established that SMARCA4/2 promote IP3R3 expression in both ovarian and lung cancer cells, likely through direct regulation of transcription.

Given the chromatin remodeling role of SWI/SNF, we then focused on the chromatin architecture of the ITPR3 locus and its potential regulation by SMARCA4/2. Indeed, SMARCA4 occupancy was observed at the ITPR3 promoter in ChIP-seq data of the BIN-67 cells upon SMARCA4 restoration (Fig. 3e)60. We also detected this SMARCA4 occupancy in H1703 cells with SMARCA4 restoration and in H1299 cells expressing inducible SMARCA461 (Fig. 3e). These data suggest that SMARCA4/2 may directly regulate ITPR3 expression. Consistent with this, we found that ChIP-seq signals of H3K27Ac, a chromatin mark associated with active promoter and enhancer, were elevated at the upstream and gene body regions of ITPR3 in BIN-67 cells after SMARCA4 restoration and in H1703 cells after restoration of SMARCA4 or SMARCA2 (Fig. 3f, upper panel). Furthermore, the assay for transposase-accessible chromatin using sequencing (ATAC-seq) peaks at these ITPR3 genomic regions were also elevated upon SMARCA4/2 restoration in H1703 cells (Fig. 3f, lower panel), indicating an enhanced chromatin accessibility at the ITPR3 locus when SMARCA4/2 were present. Together, these data suggest that SMARCA4/2 promote ITPR3 transcription by directly remodeling chromatin structure at its gene locus.

SMARCA4/2 loss inhibits apoptosis by restricting IP3R3-mediated Ca+++ flux to mitochondria. Next, we investigated whether reduced IP3R3 expression accounts for compromised Ca+++ flux in SMARCA4/2-deficient SCCOHT and NSCLC cells (Fig. 2c–n). To this end, we performed rescue experiments by suppressing SMARCA4-mediated IP3R3 induction in SCCOHT-1 and H1703 cells. Accompanied by an increase of IP3R3 levels (Fig. 4a), ectopic SMARCA4 expression in SCCOHT-1 cells strongly elevated cytosolic (Fig. 4b) and mitochondrial (Fig. 4c) Ca+++ contents in response to histamine stimulation. Notably, in these SMARCA4-restored cells, shRNA-mediated knockdown of IP3R3 to levels similar to control cells prevented ER-Ca+++ release, characterized by a significant decrease of cytosolic and mitochondrial Ca+++ contents (Fig. 4a–c). These results were confirmed in H1703 cells where suppression of IP3R3 was achieved.
by small-interfering RNA (siRNA) (Fig. 4d–f). Furthermore, cytosolic Ca\(^{2+}\) measurement upon thapsigargin stimulation in the above SCCOHT-1 and H1703 cells indicated that ER-Ca\(^{2+}\) storage capacity was not significantly altered upon ITPR3 knockdown (Supplementary Fig. 9). Together, these data indicate that reduced IP3R3 expression is the critical contributor to the compromised Ca\(^{2+}\) flux in SMARCA4/2-deficient cells.

In line with the established role of IP3R3 in Ca\(^{2+}\)-mediated apoptosis, suppression of IP3R3 in OVCAR4 cells prevented cisplatin-induced apoptosis as indicated by reduced levels of cleaved PARP and cleaved caspase 3 (Fig. 4g) and the annexin V\(^+\)/PI\(^-\) apoptotic cell population (Fig. 4h). Conversely, ectopic expression of IP3R3 in H1703 cells enhanced apoptotic induction and growth suppression after cisplatin treatment (Fig. 4i,
Supplementary Fig. 10a, b). Similarly, ectopic IP3R3 expression also sensitized BIN-67 cells to cisplatin treatment (Supplementary Fig. 10c, d). Thus, IP3R3 seems to be necessary and sufficient to mediate cisplatin-induced apoptosis in these models. Given that SMARCA4/2 directly activates ITPR3 expression (Fig. 3), we then investigated whether reduced IP3R3 expression in SMARCA4/2-deficient cells drives resistance to chemotherapy-induced apoptosis. As shown in Fig. 4j, k, while SMARCA4 restoration in H1703 cells led to increased IP3R3 expression with concomitant elevation of cleaved PARP and cleaved caspase 3 as well as the annexin V+/PI− apoptotic cell population after cisplatin treatment, knockdown of IP3R3 markedly suppressed the induction of these apoptosis markers in these SMARCA4-expressing cells, corroborating Ca2+ signaling defects in these cells (Fig. 4d–f).

Together, these data suggest that SMARCA4/2 loss inhibits chemotherapy-induced apoptosis by constricting IP3R3-mediated Ca2+ flux to mitochondria.

**IP3R3 expression is reduced in SMARCA4/2-deficient cancers.** To further validate our findings of ITPR3 regulation by SMARCA4/2 in cell models with genetic perturbation, we analyzed mRNA expression of ITPR3 and SMARCA4/2 in RNA-seq data sets of ovarian (n = 47) and lung cancer (n = 192) cell lines available from CCLE. For both cancer types, cell lines with low SMARCA4 expression (bottom quartile) also expressed lower
levels of ITPR3 compared to the rest of cell lines with high SMARCA4 expression (Supplementary Fig. 11a). Furthermore, we observed a significant positive correlation between ITPR3 and SMARCA2 in these ovarian (n = 11, r = 0.825) and lung (n = 48, r = 0.584) cancer cell lines with low SMARCA4 expression (Fig. 5a). Moreover, in a panel of 20 NSCLC cell lines, reduced ITPR3 protein was observed in SMARCA4-deficient cells compared to SMARCA4-proficient cells; overall SMARCA4/2 dual deficient cell lines expressed the lowest levels of ITPR3 (Fig. 5b). These results are in line with our above functional data, supporting that ITPR3 expression is reduced in SMARCA4/2-deficient ovarian and lung cancer cells.

Next, we investigated the relationship between ITPR3 and SMARCA4/2 expression in patient tumors. We analyzed the available TCGA RNA-seq data sets of ovarian serous cystadenocarcinoma (OV)69, LUAD, and lung squamous cell carcinoma tumors70. Similar to the above observations in cell lines, ITPR3 mRNA in patient tumors with the bottom quartile of SMARCA4 expression is significantly reduced compared to the other three expression groups in all three data sets (Supplementary Fig. 11b). Confirming the cell line results (Fig. 5a), ITPR3 was also significantly correlated with SMARCA2 mRNA in these tumors with low SMARCA4 expression (Fig. 5c, Supplementary Fig. 11c). Furthermore, we analyzed ITPR3 mRNA expression in SCCOHT patient tumors (n = 13) characterized by concomitant loss of SMARCA4/2 protein expression. In keeping with above analysis, ITPR3 mRNA in SCCOHT tumors is similar to OV tumors with low expression of SMARCA4/2 (n = 42) while significantly lower than OV tumors with high expression of SMARCA4/2 (n = 50)69 (Fig. 5d). Using immunohistochemistry (IHC), we also examined ITPR3 protein expression in patient tumors of SCCOHT and HGSCL with an ITPR3 antibody whose IHC specificity was verified by RNAi (Supplementary Fig. 12). As shown in Fig. 5e, f, SCCOHT tumors (n = 45) expressed significantly lower levels of ITPR3 than HGSCLs (n = 45). Consistently, NSCLC tumors with low SMARCA4 expression (n = 9, H-score ≤ 100) expressed significantly lower ITPR3 protein than those with higher SMARCA4 expression (n = 50, H-score > 200) (Fig. 5g, h). Together, these results from multiple cohorts of cell lines and patient tumor samples support the cooperative roles of SMARCA4/2 in regulating ITPR3 and confirm reduced ITPR3 expression in SMARCA4/2-deficient cancers.

Given that suppressed ITPR3-mediated Ca2+ flux and apoptosis has been linked to other major tumor suppressors PTEN, BAP1, and PML, in driving tumorigenesis66–68; our above analyses suggest that this may also play a role in SMARCA4/2-deficient cancers. We examined this possibility in vivo using a xenograft model of H1703 cells with exogenous SMARCA4 expression, using a validated doxycycline-controlled expression system19. Upon tumor establishment, we induced SMARCA4 expression with doxycycline treatment, which indeed resulted in suppression of tumor growth (Fig. 5i). Furthermore, IHC analysis of endpoint tumors showed that induced-SMARCA4 expression led to elevated expression of ITPR3 and cleaved caspase 3 (Fig. 5j, k). While this requires further studies, these data support that reduced ITPR3 expression in SMARCA4/2-deficient cancers may directly contribute to the tumorigenesis through suppression of apoptosis.

Histone deacetylase inhibitor (HDACi) rescues ITPR3 expression and enhances cisplatin response in SMARCA4/2-deficient cancer cells. Our data show that SMARCA4/2-deficient cancer cells are resistant to cisplatin in part through suppression of ITPR3 and that ectopic ITPR3 expression can sensitize these cancer cells to cisplatin-induced apoptosis (Fig. 4g–k, Supplementary Fig. 10). Although ITPR3 is not targetable, its expression is directly activated by SMARCA4/2 (Fig. 3). In contrast to deleterious mutations in SMARCA4, SMARCA2 loss is caused by epigenetic silencing in SCOCOH and NSCLC17,71–73. Furthermore, HDACi, a class of anti-cancer drugs that blocks the deacetylation of chromatin and other cellular substrates involved in cancer initiation and progression74–77, has also been shown to reactivate SMARCA2 expression in SCCOHT and lung cancer cells17,76,77. Indeed, treatments with quisinostat78, a second-generation HDACi, resulted in strong activation of SMARCA2 with concomitant elevation of ITPR3 at both mRNA and protein levels in SCCOHT (Supplementary Fig. 13) and SMARCA4/2-deficient NSCLC cancer cells (Fig. 6a, b). Consistent with this, quisinostat treatment strongly elevated cytosolic (Supplementary Fig. 14a) and mitochondrial (Supplementary Fig. 14b) Ca2+ contents in response to histamine stimulation, similar to the levels induced by ectopic SMARCA4 expression in H1703 cells (Supplementary Fig. 14a–c). Notably, siRNA-mediated knockdown of ITPR3 in these quisinostat-treated cells prevented ER-Ca2+ release, characterized by a significant decrease of cytosolic and mitochondrial Ca2+ contents (Supplementary Fig. 14a–c). Together, these data indicate that quisinostat treatment can indirectly restore ITPR3 expression and rescue Ca2+ flux in SMARCA4/2-deficient cancer cells.

Next, we explored the possibility of using HDACi to restore chemotherapy sensitivity in SMARCA4/2-deficient cancer cells. Indeed, the combination treatment of cisplatin and quisinostat in H1703 cells resulted in strong elevation of cleaved PARP and cleaved caspase 3 (Fig. 6b), the annexin V+PI– apoptotic cell population (Fig. 6c), and growth suppression (Fig. 6d). Given that HDACi is expected to activate expression of genes other than SMARCA2, it was important to verify the essential contribution of SMARCA2 reactivation to apoptosis induction by this drug
Fig. 5 IP3R3 expression is reduced in SMARCA4/2-deficient cancers.  

**a** Correlation of ITPR3 and SMARCA2 (A2) mRNA in ovarian (n = 11) and lung (n = 48) cancer cell lines with low expression of SMARCA4 (A4). Expression data were obtained from Cancer Cell Line Encyclopedia (CCLE) in reads per kilobase million (RPKM). A4 low, bottom quartile. r: Pearson correlation; p value (two-tailed).

**b** ImmunobLOTS of lung cancer cell lines with indicated SMARCA4/2 (A4/2) status. Pro proficient, def deficient; * KRAS mutant.

**c** Correlation of ITPR3 and SMARCA2 mRNA in ovarian cancer (n = 89) and lung adenocarcinoma (LUAD, n = 128) patient tumors with low expression of SMARCA4. Gene expression data were obtained from UCSC Xena in fragments per kilobase million (FPKM). A4 low, bottom quartile. r, Pearson correlation; p value (two-tailed).

**d** ITPR3 mRNA expression in SCCOHT and ovarian cancer patient tumors. TCGA ovarian cancers (OV) (n = 379) were stratified based on SMARCA4/2 expression as indicated in Supplementary Fig. 2b. ITPR3 expression in FPKM was normalized to ACTB. H: high; L: low. One-way ANOVA Brown–Forsythe and Welch tests followed by Dunnett’s test for multiple comparisons to A4^H, p values (p): A4^H to A2^H, p = 0.3830, A4^H to A2^L, p = 0.0009, A4^A2^L to A2^L, p = 0.0001, SCCOHT—0.0108, or two-tailed t-test between A4^A2^L group and SCCOHT, p = 0.4953. Representative images (e, g) and H-score (f, h) of immunohistochemistry analysis for IP3R3 and SMARCA4 expression in patient tumors. e, f HGSC (n = 49) and SCCOHT (n = 45). g, h NSCLC (n = 59). Scale bar, 100 μm. Mann–Whitney test (two-tailed), p values (p): f 0.0066, h 0.0270. i Tumor growth in H1703 xenograft models ± exogenous SMARCA4 expression. Doxycycline (Dox) was given daily starting on day 21 to induce SMARCA4. Upper, tumor size; lower, endpoint tumor weight. Mean ± SEM, −Dox (n = 4 animals), +Dox (n = 6 animals), two-way ANOVA (upper), two-tailed t-test (lower), p values (p): upper—0.0014, lower—0.0008. Representative images (j) and digital quantification (k) of immunohistochemistry analysis in endpoint tumors described in i. Scale bar, 100 μm. Mean ± SD, −Dox (n = 4), +Dox (n = 6), two-tailed t-test (lower), p values (p): upper, middle <0.0001; lower—0.0041. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; ns not significant.
combination. Supporting this, CRISPR/Cas9-mediated SMARCA2 knockout in H1703 cells blunted the elevation of IP3R3 and cell apoptosis markers induced by combination treatment of quisinostat and cisplatin (Fig. 6e). Furthermore, siRNA-mediated knockdown of IP3R3 also prevented the elevation of apoptosis markers in H1703 cells induced by this treatment combination (Fig. 6f).

Finally, confocal live-cell imaging demonstrated that the combination of cisplatin and quisinostat strongly induced an increase of basal mitochondrial Ca\(^{2+}\) levels in these cells (Fig. 6g, h). These results demonstrate that quisinostat can stimulate SMARCA2-dependent IP3R3 expression to restore ER-Ca\(^{2+}\) release-induced mitochondrial Ca\(^{2+}\) flux and chemotherapy sensitivity in SMARCA4/2-deficient cancer cells.

Finally, we validated the antitumor effect of this cisplatin and quisinostat drug combination in vivo using a xenograft model of H1703 cells. After tumor establishment, animals were treated with
cisplatin (4 mg kg\(^{-1}\)), quisinostat (10 mg kg\(^{-1}\)), or their combination. Consistent with our in vitro results, the combination more effectively suppressed tumor growth than each single drug alone, as indicated by a significant reduction of both tumor volume and weight (Fig. 6i). We noted that some animals treated with cisplatin or the combination, but not quisinostat alone, showed body weight loss (Supplementary Fig. 15a), likely associated with chemotherapy-induced side effects. Nevertheless, when normalized to the animal body weight, the drug combination still showed significant reduction of both tumor volume and weight compared to single treatments (Supplementary Fig. 15b, c). Furthermore, IHC analysis of endpoint tumors revealed that quisinostat treatment was able to induce protein expression of SMARCA2 and IP3R3 (Fig. 6j, k) and, when combined with cisplatin, synergistically elicited a strong apoptotic response as indicated by a marked increase of cleaved caspase 3 levels (Fig. 6j, k). Taken together, our data provide a proof-of-concept treatment strategy for enhancing chemotherapy response in patients affected by SMARCA4/2-deficient cancers.

**Discussion**

We show that SMARCA4/2 deficiency impairs chemotherapy-induced apoptotic responses in ovarian and lung cancers at least in part by altering ER to mitochondria Ca\(^{2+}\) flux. By directly restricting ITT3R3 expression, SMARCA4/2 loss inhibits Ca\(^{2+}\) transfer from the ER to mitochondria required for apoptosis induction. Consequently, stimulation of ITT3R3 expression through SMARCA2 reactivation by HDACi enhanced chemotherapy response in SMARCA4/2-deficient cancer cells.

SWI/SNF subunits are frequently mutated in human cancers\(^4\), which has been connected to hallmarks of cancers including aberrant cell proliferation, lineage differentiation, and altered metabolism\(^1,2,8\). Our findings establish a functional link between SMARCA4/2 loss and dampened ITT3R3-mediated Ca\(^{2+}\) flux in resisting programmed cell death. While our current study mostly focuses on chemoresistance, we also found that SMARCA4 restoration alone suppressed tumor growth of H1703 xenografts associated with increased expression of ITT3R3 and cleaved caspase 3. This suggests that altered Ca\(^{2+}\) homeostasis may also directly contribute to the tumorogenesis of SMARCA4/2 loss through suppression of apoptosis, as previously shown for other major tumor suppressors PTEN, BAP1, and PML\(^{66–68}\). Additional investigations are warranted to further confirm these results. Given the cooperative roles of SMARCA4/2 in regulating ER to mitochondria Ca\(^{2+}\) flux and apoptosis, it is likely that they exert these functions in a SWI/SNF-dependent manner. Therefore, exploring the potential role of other SWI/SNF subunits frequently altered in cancers, such as ARID1A\(^4\), in Ca\(^{2+}\) homeostasis and apoptosis may help understand the oncogenic mechanisms underlying other SWI/SNF-deficient cancers.

Our study examined the roles of SMARCA4/2 in regulating chemotherapy response and apoptosis induction using cancer cell lines that naturally harbor SMARCA4/2 alterations. This is different from previous studies employing RNAi-mediated SMARCA4 knockdown in SMARCA4-proficient cancer cells which led to enhanced response to DNA damaging agents\(^{31–33}\). We found that naturally occurred SMARCA4/2-deficient cancer cells are more resistant to chemotherapy, which is in line with previous reports showing that SCCOHT is typically more resistant to conventional chemotherapy in both cell models and patients\(^{15,34}\). Similarly, experimental suppression of SMARCA2 has been shown to be selective lethal to SMARCA4-deficient/SMARCA2 proficient cancer cells\(^{18–20}\). However, concomitant loss of SMARCA4/2 occurs in almost all SCCOHTs and a subset of NSCLCs associated with poorer prognosis in patients\(^{6,10,16,17}\). Therefore, naturally occurred SMARCA4/2-deficient cancers may represent a unique group with distinct properties such as altered Ca\(^{2+}\) homeostasis leading to chemotherapy resistance.

Similar to SCCOHT, our analysis in multiple NSCLC datasets of diverse tumor staging including the most comprehensive Director’s Challenge data set suggests that reduced SMARCA4 expression is associated with chemoresistance in NSCLC. A previous report\(^{79}\) analyzing the JBR.10 data set of NSCLCs from early stages\(^{80}\) showed that patients whose tumors expressed low SMARCA4, but not high SMARCA4, benefited from the adjuvant therapy of cisplatin and vinorelbine (a microtubule inhibitor). This discrepancy is likely due to differences in SMARCA4 microarray probe sets chosen, patient cohort compositions, and data analysis methods. While we used the optimal “jetset probe” unbiasedly identified by the KM plotter without preassociation with patient outcome, microarray technology has limited sensitivity and specificity in quantifying gene expression. Thus, these results require further confirmation using better tools such RNA-seq. In addition, we recognize that patient outcome is often influenced by multiple factors such as treatment history, which was not uniform among all patients analyzed. Therefore, additional clinical studies are needed to better control these variants and evaluate roles of SMARCA4/2 expression in predicting chemotherapy responses in NSCLCs.

HDACi have been clinically approved for the treatment of several hematological malignancies but their activity in solid tumors has been limited as single agents\(^4,73\). Thus, identifying genetic vulnerability of HDACi and effective drug combinations
may enhance their clinical utility. SCSOHT cells have been shown to be more sensitive to HDACi than SMARCA4/2-deficient cancers. This may be because NSCLC have a more complex genetic make-up than SCSOHT. Our study provided proof-of-principle data supporting that HDACi may be a potential therapeutic strategy to stimulate IP3R3 transcription through SMARCA2 reactivation and sensitize SMARCA4/2-deficient cancers to chemotherapy. Other strategies may also be explored. For example, GGTi-2418, a geranylgeranyl transferase inhibitor, sensitizes A549 cells to apoptosis induction by photodynamic therapy both in vitro and in xenograft models via stabilizing the IP3R3 protein. Of note, A549 is also a SMARCA4-deficient NSCLC cell line and this independent study does further support the notion of elevating IP3R3 expression to enhance chemotherapy response in SMARCA4/2-deficient cancers. However, both HDACi and GGTi-2418 intervene IP3R3 expression indirectly and may cause unexpected toxicity. Therefore, other agents that directly facilitate Ca2⁺ flux from the ER to mitochondria need to be investigated in the future. In addition to IP3R3, other common targets of SMARCA4/2 may also play a role in altered Ca2⁺ homeostasis impacting apoptosis, which could serve as potential drug targets in SMARCA4/2-deficient cancers and will require further studies.

In summary, we have uncovered that SMARCA4/2 loss restricts IP3R3-mediated Ca2⁺ flux from the ER to mitochondria, leading to resistance to chemotherapy-induced apoptosis in ovarian and lung cancers. Our study provides insights into the molecular mechanisms of SWI/SNF loss in promoting drug resistance and suggests a potential therapeutic strategy to enhance chemotherapy response in patients affected by SMARCA4/2-deficient cancers.

Methods

Cell culture. All cell lines were cultured in Roswell Park Memorial Institute 1640 Medium (Thermo Fisher Scientific, Cat# 11875-093) with 7% fetal bovine serum (Sigma, Cat# F1051), 1% penicillin/streptomycin (Thermo Fisher Scientific, Cat# 25300-081), except for 293T with Dulbecco’s modified Eagle medium (Thermo Fisher Scientific, Cat# 11995-065) and HEK293 with Eagle’s minimum essential medium (Wisent, Cat# 320-005-CL). Cells were maintained at 37°C and 5% CO₂ and regularly tested for Mycoplasma using Mycoalert Detection Kit (Lonza, Cat# LT07-318). All cell line origins are listed in Reporting Summary and have been validated by short tandem repeat analysis.

Lentivirus production and infection. All experiments with ectopic expression, shRNA knockdown, and CRISPR single guide RNA (sgRNA) knockout were performed using lentiviral production. For 2 × 10⁵ 293T cells were plated in 2 mL of DMEM medium per well in a six-well plate and transfected after ~8 h with lentiviral constructs, the packaging (psPAX2), and envelope plasmid (pMD2.G) by CaCl₂. Virus containing medium were harvested at 24 and 36 h after transfection before use or stored at −80°C. For infection, ~5 × 10⁵ target cells were plated the day before and infected with virus for ~8 h. After ~20 h recovery, cells were selected in medium containing 2 μg/mL puromycin or 5 μg/mL blasticidin for 2–3 days and harvested for the experiments.

Compounds and antibodies. Caspatin (S1166), quinostatin (S1096), pacitaxel (S1150), and topotecan (S9321) were purchased from Selleck Chemicals. Cyclophosphamide (CA80500-080), histamine (H7125), and thapsigargin (T9033) were from Sigma-Aldrich. Mitotracker deep red FM was from Thermo Fisher Scientific (M22420). Antibodies against calsequestrin (Cat# sc-66737), HSP90 (Cat# 13119), and β-Actin (Cat# sc-7778) were from Santa Cruz Biotechnology; antibodies against SMARCA2 (Cat# 11996), cleaved PARP (Cat# 5625), and cleaved caspase 3 (Cat# 9664) were from Cell Signaling; antibodies against MICU2 (Cat# ab-101465), VDAC1 (Cat# ab-14734), and GRP78 (Cat# ab-2799) were from Abcam; antibody against SMARCA4 (Cat# A300-813A) was from Bethyl Laboratories (A300-813A); antibody against IP3R3 (Cat# 610312) was from BD Pharmingen; antibody against vinculin (Cat# V4050) was from Sigma-Aldrich; antibody against MCU (Cat# HPA0168480) was from Atlas; and antibody against MICU1 (Cat# orb-323178) was from Biorbyt. Antibody against SMARCA4 was used with 1:5000 dilution and all others with 1:1000 dilutions. Antibodies for IHC are described in the Immunohistochemistry method section below. Secondary antibodies goat anti-mouse IgG (Cat#1706516) and goat anti-rabbit IgG (Cat#1706515) were purchased from Bio-Rad.

Plasmids. Individual shRNA vectors used were from the Mission TRC library (Sigma) provided by McGill Platform for Cellular Perturbation (MCP) of Rosa- liud and Morris Goodman Cancer Research Centre and Biochemistry at McGill University: shSMARCA2#1 (TRCN0000358828); shSMARCA2#2 (TRCN0000203033); shITPR3#1 (TRCN0000613242); and shITPR3#2 (TRCN000061326). For shRNA experiments, pLKO vector control was used. Scramble control sgRNA (SCR_6) and dual-sgRNAs targeting SMARCA4 (TEID=1074696) were from the RNPEDIT CRISPR Library (Transom) provided by MOPC. Additional sgRNA (GCTGGGGGAGG ATGTTTCGCCG) targeting SMARCA4 was cloned into pLentiCRISPRv2. pReceiver control vector, pReceiver-SMARCA4, and pReceiver-SMARCA4 were provided by Dr. Jannik N. Andersen (The University of Texas, MD Anderson Cancer Center).

CRISPR/Cas9 editing. Plasmid-based CRISPR/Cas9 editing was used to generate SMARCA4 knockout in OVCA4 and H1437 cells using standard lentiviral delivery followed by single-cell cloning. For HEK116 cells, ribonucleoprotein complexes were used. RNA targeting SMARCA4 (sequence = GCCGTTGGCATCACGGGCG) and tracrRNA duplexes (1 μM) were formed by heating at 95°C and gradual cool down to room temperature (RT). RNPs were formed by combining the 1 μM guide RNA oligos with 1 μM Alt-R p.555, R.555, and 30 nM Alt-R p.555. Transfection complexes containing these RNA complexes and Lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific) were diluted in Opti-MEM media and incubated at RT for 20 h. H1437 endometrial cancer cells were transiently transfected in the wells of a 24-well tissue culture plate to achieve a final concentration of 40,000 cells/well and the number of total colony forming units (CFUs) at 20 nM cytometry (flow cytometry) was quantified. Flow Cytometry Facility was utilized to enrich for CRISPR transfected cells positive for tracrRNA-ATTO™ 550 fluorescence. Single clones were either generated by flow cytometry plating a single cell per well into a 96-well plate or manually plating of 0.5 cells/well into a 96-well plate upon filtration through a cell strainer. Single-cell-derived clones were validated by Sanger sequencing over the guided nuclease target region.

CRISPR sgRNA screen. The transEDIT™ pCLIP-All-EFS-Puro Epigenetics CRISPR Screening library (Transom) containing 5080 sgRNAs targeting 496 epigenetic genes was used in this study. Library virus was generated in 293T cells as described above. OVCAR4 cells were infected with library virus at low multiplicity of infection achieving single sgRNA integration. After 48 h, ~5 × 10⁵ cells were plated the day before and infected with virus for ~8 h. After ~20 h recovery, cells were selected in medium containing 2 μg/mL puromycin or 5 μg/mL blasticidin for 2–3 days and harvested for the experiments.

 Colony formation assays. Since different cell lines have variable proliferation rates and sizes, plating densities for each line were first optimized to allow about two weeks of drug treatment, before cells reach 90% confluency in six-well plates. Single-cell suspensions of all cell lines were then counted and seeded into six-well plates with the densities predetermined (2 × 10⁴ cells/well). Cells were treated with vehicle control or drugs on the next day and culture medium was refreshed every 3 days for 10–14 days in total. At the endpoints of colony formation assays, cells were fixed with 3.75% formaldehyde, stained with crystal violet (0.1%w/v), and photographed. All relevant assays were performed independently at least three times.

 Cell viability assays. Cultured cells were seeded into 96-well plates (1,000–6,000 cells/well). Twenty-four hours after seeding, different dilutions of compounds were added to cells. Cells were then incubated for 4 days and cell viability was
measured using the CellTiter-Blue Viability Assay (Promega) by measuring the fluorescence (560/590 nm) in a microplate reader. Relative survival in the presence of drugs was normalized to the untreated controls after background subtraction.

Protein lystate preparation and immunoblots. Cells were first seeded in normal medium without inhibitors. After 24 h, the medium was replaced with fresh medium containing the inhibitors as indicated in the text. After the drug stimulation, cells were washed with cold PBS, lysed with protein sample buffer, and processed with Novex NuPage GE Cell Electrophoresis Systems (Thermo Fisher Scientific), HSP90, vinculin, or calreticulin were used as loading controls. The quantification of immunoblots was performed on two independent experiments using Image J and normalized to loading controls are displayed in Supplementary Fig. 16. Uncropped immunoblots presented in main figures are displayed in Supplementary Fig. 17. Uncropped immunoblots presented in Supplementary Figures are included in Source Data.

Annexin V staining and IncuCyte imaging. Cells in 96-well plates were treated with cisplatin at different concentrations in medium containing IncuCyte annexin V for apoptosis (Essen Bioscience, Cat# 4641). IncuCyte live-cell imaging system was used to record four images every 2–4 h. Images were analyzed by IncuCyte Zoom (2016B) software and annexin V positive cells were normalized to phase-contrast confluency for each well.

Annexin V and PI flow cytometry. For apoptosis assays, negative controls were prepared by incubating cells in the absence of inducing agent and positive controls for apoptosis were prepared using 10 μM H2O2. Cells were harvested after treatment and washed in cold phosphate buffered saline and resuspended in 1× annexin binding buffer (BMS500BB) to 10^6 cells/mL. One hundred microliters of cell suspension was used per assay and 5 μL of FITC annexin V (A13199) and 1 μL of PI (P3040MP) diluted to 100 μg/mL in annexin V binding buffer was added to each suspension. Cells were incubated following addition of fluorescent reagents for 15 min at RT. Four hundred microliters of 1× annexin V binding buffer was added to each suspension following incubation and the samples were mixed gently and kept in the dark and on ice prior to analysis. Flow cytometry was performed using Guava easyCyte HT (Sigma) with the guavaSoft 2.5 software (Sigma) based on the manufacturer’s instructions. Fluorescence emission was measured at 530 and >575 nm to separate between the green and red populations. Technical controls for gating were prepared on undrugsed cells with both PI and annexin V stains, with either PI or annexin V only, or in the absence of both. Apoptotic cell population (annexin V^+/PI^-) showed green fluorescence only. Gating strategy is exemplified in Supplementary Fig. 18.

RNA isolation and real-time quantitative reverse transcription PCR (qRT-PCR). After indicated drug treatment or genetic modifications, cells were harvested for RNA isolation using Trizol (Thermo Fisher Scientific, Cat # 15596018) the next day. Synthesis of cDNA was performed using Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Cat# K1642) and qRT-PCR assays using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Cat# A25742) were carried out according to the manufacturer’s protocols. Relative mRNA levels of each gene shown were normalized to the expression of the housekeeping gene GAPDH. The sequences of the primers are listed in Supplementary Table 3.

Survival analysis. Survival analyses were performed on LUAD patients from the following datasets with available information on adjuvant chemotherapy status: Director’s Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma 47, KM plotter 48, and The UT Lung SPORE (GSE117735, RNA-seq), and from Lung Cancer RNASeq (GSE117735, RNA-seq). Processed gene expression data were obtained from original study for GSE117735 and by GEOquery (2.5.6) 49 for GSE109010. For all A25742) were carried out according to the manufacturer’s recommendations.

Intracellular Ca^2+ measurements. To measure cytosolic or mitochondrial Ca^2+, OVCAR4, H1703, SCOCC0-1, and H1437 cells were cultured on Nunc Lab-Tek chambered eight-well cover glass (Thermo Fisher Scientific, cat# 154534) and transiently transfected with the cytosolic R-GECO1 (Addgene, cat# 32444) or mitochondrial CEP1A-2mt (Addgene, cat# 58210) reporter probes. Cells were washed three times in a balanced salt solution buffer + Ca^2+ (BSS) (120 mM NaCl, 5.4 mM KCl, 0.8 mM MgCl_2, 5 mM NaHCO_3, 6 mM HEPES [pH 7.3]). Fluorescence values were then collected every 2 s for 30 s. ER-Ca^2+ release was stimulated by injection of 100 μM histamine final in BSS + Ca^2+ at 10 s. Images were acquired using a 40× objective of the Nikon Eclipse Ti-E microscope, coupled to an Andor Dragonfly spinning disk confocal system equipped with an Andor Ion camera, exciting with 488 nm or 561 nm laser for CEP1A-2mt or R-GECO, respectively.

To measure total ER-Ca^2+ content, OVCAR4, H1703, SCOCC0-1, and H1437 cells were cultured on Nunc Lab-Tek chambered eight-well glass cover and transiently transfected with the cytosolic R-GECO2 Ca^2+ reporter probe. Cells were washed three times in a BSS solution buffer + Ca^2+ (BSS) (120 mM NaCl, 5.4 mM KCl, 0.8 mM MgCl_2, 5 mM NaHCO_3, 6 mM HEPES [pH 7.3]). Fluorescence values were then collected every 2 s for 5 min. ER-Ca^2+ release was stimulated by injection of 10 μM thapsigargin final in BSS-Ca^2+ at 10 s. Images were acquired using a 40× objective of the Nikon Eclipse Ti-E microscope, coupled to an Andor Dragonfly spinning disk confocal system equipped with an Andor Ion camera, exciting with 488 and 647 nm lasers for CEP1A-2mt and Mitotracker deep red, respectively.

Immunohistochemistry. Tissue microarrays (TMAs) of tumor samples of HGSC and NSCLC patients used in this study were previously described 48,49. A TMA of 52 SCOCC0 patient tumors was constructed for this study. Studies on SCOCC0 patient tumors were approved by the Institutional Review Board (IRB) at McGill University.
University, McGill IRB # A08-M61-09B. Studies on 59 pathologist-confirmed ovarian HGSC samples were approved by the ethics boards at the University. Hospital for Sick Children and Jewish General and informed consent was obtained from all participants in accordance with the relevant IRB approvals. Hematoxylin and eosin-stained sections of the 50 SCCOHTs (confirmed by DNA mutation analysis or/and SMARCA4 IHC) and 32 HGSC cases were reviewed and areas containing tumor only were demarcated and scored to construct TMAs using duplicate 0.6-mm cores from the demarcated areas. A panel of 100 reviewed LSAD patient tumors were analyzed. This study was approved by the ethics boards at the McGill University Health Centre (F11HR, 17212). The NSCLC TMA was comprised of 4 mm cores from the selected paraffin-embedded tissue blocks. For all IHC analysis, cores with low tumor cellularity and artifacts were not included in the analysis.

The 4 μm thick sections from these TMAs were cut, deparaffinized, and stained using the BenchMark Ultra system (Ventana Medical Systems Inc). Heat-induced epitope retrieval (HIER) was performed with Ultra Cell Conditioning Solution (CC1) for 64 min at 95 °C, followed by 16 min of incubation at 36 °C with the rabbit monoclonal antibody against SMARCA4 (clone EPNCIR1111A; dilution, 1:100; Abcam). For IP3R3, HIER was performed in CC1 for 48 min at 100 °C followed by incubation for 48 min at 36 °C with the mouse anti-IP3R3 (BD Transduction Laboratories). After primary antibody incubation, detection was performed using the default OptiView DAB protocol as per the manufacturer’s directions (Ventana). The slides were digitalized using an Aperio scanner and the Lumenera INFINITY X CMOS camera.

For patient tumors, assessment of SMARCA4, unequivocally absent staining in the nuclei of viable tumor cells as opposed to strong staining in background stromal cells was considered IHC negative. Expression in the tumor cells that is equivalent to the staining of nonproliferating cells in the background was considered IHC positive. Cells were analyzed according to the staining intensity on a scale of 0–3 (0 = negative, 1 = weak, 2 = moderate, 3 = strong). H-scores were calculated as the sum of the percent of cells at each intensity (Pi) multiplied by the intensity score (i). H-score = Σ(Pi*i) x 100. Score values range between 0 and 300. Cytology samples and artifacts were not included in the analysis.

For xenograft tumor sections, quantification of percentage positive staining for SMARCA4, SMARCA2, and cleaved caspase 3 was performed unbiasedly using the Aperio nuclear algorithm on Aperio ImageScope. Quantification of percentage positive staining for IP3R3 was performed unbiasedly using the Aperio cytoplasm algorithm on Aperio ImageScope. Weak IP3R3 (+) staining resulting from the background was not considered in the analysis.

Mouse xenografts and in vivo drug studies. Animal experiments were performed according to standards outlined in the Canadian Council on Animal Care Standards (CCAC) and the Animal Care Committee, R.S.O. 1990, Chapter c. A.22, and by following internationally recognized guidelines on animal welfare. All animal procedures (Animal Use Protocol) were approved by the Institutional Animal Care Committee of the CCAC. All animal experiments were carried out under the guidance of a veterinary pathologist trained in animal care and welfare. Animal experiments were performed using 8–12-week-old in house bred male NOD.Cg-Pkd-cdelhang Iizra1M1Wf1/SjI (NSG) mice.

For in vivo drug studies, Quisinostat (SelleckChem) was resuspended in 20% hydroxypropyl-β-cyclodextrin (Sigma-Aldrich) buffer (pH = 8.70) at a concentration of 50 mg/mL−1 (administered intraperitoneally at 10 mg kg−1 dose for a 25–28 g mouse) and was administered twice per week. Cisplatin (SelleckChem) was resuspended in 0.9% sodium chloride solution (administered intraperitoneally at 4 mg kg−1 dose for a 25–28 g mouse in a volume of 200 µL). These two reagents were stored at −80 °C. Tubes were thawed overnight at 4 °C.

For the tumor model, single-cell suspension was created by dissociating a sufficient number of subconfluent flakes of cells to produce 4 × 105 cells (H713 or H703 expressing pN20-SMARCA4) in 200 µL of Matrigel HC in a 50:50 ratio (Corning Matrigel HC, VWR). The tumor cell suspension was subcutaneously injected into the left flank of each NSG mouse. For the doxycycline inducible model using H713 cells expressing pN20-SMARCA4, as tumor volumes (V = (H × W2)/2) reached 150 mm3 (21 days post inoculation), experimental mice (n = 6) were injected with 2.5 mg/mL doxycycline (Millipore Sigma) intraperitoneally followed by 2 mg/mL in sucralose (MediDrop, ClearH2o) solution ad libitum. Experimental animals were observed weekly for a week prior to the experiment to acclimatize mice to the taste. For the chemo drug treatment experiment, when tumor volumes reached 150 mm3 days post inoculation), which was assigned as day 0, the mice were entered into the treatment regimen (21 days). Mice were randomly allocated to control (vehicle, n = 6), quinostatin (10 mg kg−1 quinostatin, once per three times per week, n = 5), cisplatin (4 mg kg−1 cisplatin, once per week, n = 5), or combination (10 mg kg−1 quinostatin and 4 mg kg−1 cisplatin, n = 5) group. Mice were housed in groups of 4–5, with each group consisting of both vehicle control and treatment animals matched for tumor size on day 0 of treatment. Tumor progression was monitored and measurements using digital calipers (VWR) were recorded twice weekly. The persons performing all the tumor measurements and the IHC analysis for the endpoint tumor samples were blinded to the treatment information.

Statistics and reproducibility. GraphPad Prism 8 software was used to generate graphs and statistical analyses. Methods for statistical tests, exact value of a, and all the tumor measurements and the IHC analysis for the endpoint tumor samples were blinded to the treatment information.

Data availability
Original data for IC50 of chemotherapy drugs are available from GDSC (https://www.cancer.gov/). mRNA expression data of SMARCA4/2 and ITPR3 are available from the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle) for cell lines and from UCSC Xena (https://xenabrowser.net/datapages/) for TCGA tumors of lung and ovarian cancer patients. Out of 13 SCCOHT patient tumors, RNA-seq data of ten cases were obtained from a previous study67 and that of the other three cases can be found using the accession number EGA000010005484. Source data for RNA-seq, microarray, ChiP-seq, and ATAC-seq can be found using the accession number GSE120979G, GSE117359G, GSE121755G, GSE109010, and GSE10902061. All unique materials generated are readily available from the authors. All other data supporting the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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Competing interests

The authors declare no competing interests.

Additional information

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