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Abstract

Many cancers evade immune rejection by suppressing MHC-I antigen processing and presentation (AgPP). Such cancers do not respond to immune checkpoint inhibitor therapies (ICIT) such as PD-1/PD-L1 [PD-(L)1] blockade. Certain chemotherapeutic drugs augment tumor control by PD-(L)1 inhibitors through potentiation of T cell priming but whether and how chemotherapy enhances MHC-I dependent cancer cell recognition by cytotoxic T cells (CTL) is not entirely clear. We now show that the lysine acetyl transferases p300/CBP control MHC-I AgPPM expression and neoantigen amounts in human cancers. Moreover, we found that two distinct DNA damaging drugs, the platinoid oxaliplatin and the topoisomerase inhibitor mitoxantrone, strongly upregulate MHC-I AgPP in a manner dependent on activation of NF-κB, p300/CBP and other transcription factors, but independently of autocrine IFNγ signaling. Accordingly, NF-κB and p300 ablations prevent chemotherapy-induced MHC-I AgPP and abrogate rejection of low MHC-I expressing tumors by re-invigorated CD8$^+$ CTL. Drugs like oxaliplatin and mitoxantrone may be used to overcome resistance to PD-(L)1 inhibitors in tumors that had "epigenetically downregulated", but had not permanently lost MHC-I AgPP activity.

Significance Statement

T cells recognize their targets via their T cell receptors (TCR), which in the case of CD8$^+$ T cells bind to MHC-I:antigen complexes on the surface of target cells. Many cancer cells evade immune recognition and killing by downregulating MHC-I AgPPM. Here, we show how the histone acetyl transferases P300/CBP together with NF-κB epigenetically regulate expression of MHC-I molecules, immunoproteasome subunits and peptide transporter to enable proper MHC-I antigen presentation. Notably, this pathway is frequently disrupted in human cancers. We now show that certain chemotherapeutics can augment MHC-I antigen presentation via NF-κB and p300/CBP activation thereby enhancing cancer cell recognition and killing by effector CD8$^+$ CTLs.

Main Text

Introduction

Immune checkpoint inhibitor therapy (ICIT) had transformed cancer treatment (1–4), but even in ICIT-responsive metastatic melanoma and non-small cell lung cancer (NSCLC), response rates rarely exceed 40% (5). Other malignances, including prostate cancer (PCa) and pancreatic ductal adenocarcinoma (PDAC), are ICIT refractory (6–9). For a given neoplasm to respond to immune checkpoint inhibition, in particular PD-(L)1 blockade, it needs to be populated by CTL that recognize tumor antigens (4). However, even CTL populated tumors can evade immune elimination either through activation of immunosuppressive mechanisms that induce CD8$^+$ T cell suppression or restrain their entry into tumors (10), down-regulation of MHC-I AgPP (11, 12) or antigen editing and loss (13). Various strategies have been used to enhance ICIT responsiveness, including induction of immunogenic cells death (ICD) by radiotherapy and chemotherapy (14). By enhancing the release of damage associated molecular patterns (DAMP) and other molecules, ICD stimulates tumor antigen uptake by antigen-presenting cells (APC) that prime T cells against tumor antigens, as demonstrated by vaccination experiments (15). Primed T cells may accumulate in the tumor and lead to immune rejection as long as they can recognize and kill their targets (16). Such strategies are ineffective in cancers with low MHC-I or HLA-A/B/C expression (11, 16–19) .

PCa is a typical ICIT-refractory cancer, presumably due to low expression of HLA-A/B/C molecules that together with β2 macroglobulin form MHC-I heterodimers, which present tumor antigens to CD8$^+$ CTL (20, 21). Using mouse models of PCa we found that the platin-based DNA-crosslinker oxaliplatin (Oxali) potentiates immune rejection of autochthonous or engrafted tumors after genetic or pharmacological depletion of PD-L1-expressing immunosuppressive IgA$^+$ plasmocytes, which cause CTL exhaustion (22). Low dose Oxali also enhances mouse PCa regression in response to anti-PD-L1 treatment (22). Similar results were obtained with low dose Oxali or photodynamic therapy in other cancer models (23, 24) but the underlying mechanisms have not been explored. As Oxali is known to induce ICD and T cell priming, we investigated whether its ability to potentiate the immune rejection of IgA$^+$ plasmocyte- depleted or anti-PD-L1 treated low MHC-I prostate tumors also entails effects on the recognition and killing step of the cancer immunity cycle, which depends on CTL-MHC-I interactions (16, 25). Here we show that Oxali and the structurally unrelated topoisomerase II inhibitor mitoxantrone (Mito) transcriptionally upregulate expression of MHC-I molecules and their cognate antigen presentation and processing machinery (AgPPM). This response, which takes place in human and mouse cancers, depends on activation of NF-κB and nuclear translocation of the closely related histone (and lysine) acetyl transferases p300 and CREB binding protein (CBP). Whereas p300 ablation abrogated MHC-I AgPP induction and the synergy between low dose Oxali and PD-(L)1 blockade, it had no effect on induction of anti-tumor immunity by Oxali-killed PCa cells used as an immunogen.

Results

Oxaliplatin and mitoxantrone induce MHC-I AgPPM genes

To determine the effect of Oxali and related drugs on gene expression in PCa cell lines used in our previous study (22), Myc-CaP cells were treated with different drugs at doses that induce no more than 10 % cell death, and vital cells (SI Appendix, Fig. S1A and S1B) were analyzed by whole genome RNA sequencing (RNAseq) and ATAC-seq (Assay for Transposase-Accessible Chromatin) (26). Since CTL re-invigoration by anti-PD-L1 induces IFNγ production (27, 28), we also examined the effect of IFNγ alone or together with chemotherapy. Low-dose chemotherapy, in particular Oxali, induced marked changes in gene expression and chromatin accessibility depicted as Differentially Expressed Genes (DEG) and Differentially Accessible DNA Regions (DAR) (Fig. 1A and SI Appendix, Fig. S1C-S1F). The platinoid-induced changes were usually augmented by IFNγ, although the effects of Oxali were broader than that of

IFNγ, which mainly enhanced gene expression magnitude rather than breadth. Some of the Oxali or IFNγ inducible gene sets were common to both agents (Fig. 1A). Pathway enrichment analysis (Fig. 1B) identified the most significantly enriched pathways, activated by Oxali (red, e.g. Epithelial-mesenchymal transition, TP53), IFNγ (blue, e.g. Myc) or Oxali + IFNγ together (purple, e.g. IFN type I and II, AgPPM). Notably, while either Oxali or IFNγ significantly enriched genes involved in MHC-I AgPP and IFNγ signaling, these effects were strongly enhanced when Oxali and IFNγ were combined (Fig. 1B and SI Appendix, Fig. S2A-S2C). However, Oxali did not induce IFNγ expression, indicating that its ability to induce MHC-I AgPPM components was not due to autocrine IFNγ signaling.

Pathway enrichment analysis of DEGs that were responsive to Oxali plus IFNγ revealed strong induction of genes related to type I and II IFN signaling and MHC-I AgPPM components, involved in protein folding, MHC-I complex assembly and peptide loading, as well as genes involved in the ER-phagosome pathway and anti-viral responses (Fig. 1C and SI Appendix, Fig. S2D). Most of these genes were also induced by Oxali alone. To understand how these genes were induced we examined the ATAC-seq patterns of a gene cluster on mouse chromosome 17 harboring the *Psmb9*, *Tap1*, *Psmb8*, and *Tap2* genes, coding for immunoproteasome components and peptide transporters (SI Appendix, Fig. S1E-S1F and S3A). Low dose Oxali, and to a lesser extent Cisplatin (Cispl), increased transcription factor (TF) accessibility at several sites within this locus (SI Appendix, Fig. S3A). Surprisingly, IFNγ alone had little effect, if any, on chromatin structure (SI Appendix, Fig. S1F). Q-RT-PCR analysis further confirmed induction of AgPPM genes by Oxali and Cispl and to a lesser extent by Mito, alone or together with IFNγ (SI Appendix, Fig. S3B-S3D). A similar response pattern was displayed by the *Nlrc5* gene coding for NLRC5/CITA, the master activator of the MHC-I AgPPM (Fig. 2A). ATAC-seq revealed increased *Nlrc5* chromatin accessibility after low dose Oxali or Cispl , but hardly any change after IFNγ treatment (Fig. 2B). Mito, Cispl and Oxali, but not IFNγ, induced *Ifngr2* mRNA, but had little effect on chromatin accessibility of its gene (Fig. 2C-2D). Of note, the chromosome 17 region opened up by Oxali contains binding motifs recognized by BORIS and CTCF (SI Appendix, Fig. S3A), general TF responsible for chromatin opening (29). Low dose Oxali also increased β2 microglobulin (β2M) and all tested chemotherapeutics induced surface and mRNA expression of H-2Kq, the predominant MHC-I molecule in Myc-CaP cells (Fig. 2E-2F and SI Appendix, Fig. S3D). Low-dose Oxali increased immunoproteasome activity measured with an LMP7/PSMB8-specific substrate, Ac-ANW-AMC, an effect that was potentiated by IFNγ (Fig. 2G).

Putative transcriptional regulators of MHC-I AgPPM induction
We searched for signaling pathways and TF mediating MHC-I AgPPM and IFNγR2 induction by low dose Oxali. RNA-seq and pathway enrichment analyses suggested involvement of IRF, STAT, NF-κB, MYC family members and androgen receptor (AR) (Fig. 2H-2K, and SI Appendix, Fig. S3E-S3F). Whereas the IRF, STAT and NF-κB pathways were upregulated by Oxali and potentiated by IFNγ, the MYC and to a lesser extent the AR pathway, both of which participate in PCa tumorigenesis (30–32), were downregulated after Oxali + IFNγ treatment. Amongst IRF family members, IRF1, 7 and 9 were stimulated by Oxali and IFNγ, IRF2 was induced by Oxali and IRF8 mainly responded to IFNγ (SI Appendix, Fig. S3F). Similarly, STAT1 and 2 were stimulated by Oxali, whereas IFNγ induced STAT1 and 3. JUN, ATF3, UBA7, CREB3, NFE2L1 and SOCS1 were induced by low-dose Oxali, along with NF-κB1 (p105) and NF-κB2 (p100) (SI Appendix, Fig. S3F). ATAC-seq confirmed that Oxali, but not IFNγ, enhanced chromatin accessibility of the *Nfkb1* locus (Fig. 2H).

We employed two additional analytic approaches to identify master TF mediating treatment-induced expression changes [LISA (33)] and TF binding enrichment within regions of differential chromatin accessibility [GIGGLE (34)]. These analyses predicted the master regulators (MRs) most likely to influence the DEGs (Fig. 2I) and DARs (Fig. 2J) by leveraging the complete set of TF binding datasets

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available from the CistromeDB collection. The results further highlighted treatment-related directional TF associations (up/downregulated DEGs, open/closed DARs); Oxali: ESR1, NR3C1 and JUN; IFNγ: MYC, STAT1, ATF4 and FOS; Oxali + IFNγ: STAT1, YAP1, ESR1, IRF1, RELA and IRF8 (Fig. 2I-2J). DEG and DAR integration revealed 200 common TF, including IRFs and STATs (Fig. 2K). Notably, Oxali treatment elicited marked changes in histone methylation- and acetylation-related gene signatures (Fig. 2L) in agreement with the ATAC-seq data (SI Appendix, Fig. S1E-S1F). Subset analyses focusing on histone modifying factors implicated the involvement of the histone acetyltransferases (HATs) p300 and CBP and several histone deacetylases (HDACs) (SI Appendix, Fig. S4A).

To confirm induction and/or activation of some of the above TF, PCa (Myc-CaP, TRAMP-C2/TRC2) and colon cancer (MC38) cell lines were treated as above with or without IFNγ. Protein immunoblotting (IB) and flow cytometric analyses confirmed induction of ER stress (P-eIF2α, CHOP) and DNA damage (p-p53, γH2AX, p-ATM) markers, IRFs (IRF1, p-IRF3, IRF7), STATs (P-STAT1 and STAT1), CREB1, JUNB, type I IFN inducing proteins (cGAS, STING), PSMB9, and NF-κB signaling components (IκBα, RELA/p65, P-p65) (SI Appendix, Fig. S4B-S4G).

Chemotherapy stimulates HAT nuclear localization and activity
Chromatin structure opening, as revealed by ATAC-seq analysis, depends on histone acetylation (35). Importantly, Oxali treatment of Myc-CaP cells, increased HAT enzymatic activity within 3 h and its effect was comparable to that of an HDAC inhibitor (HDACi) (Fig. 3A-3B). Oxali and Mito also increased total p300, acetylated CBP/p300 and K310-acetylated RELA/p65 nuclear amounts (Fig. 3C). IFNγ also increased nuclear p300, but its effect was considerably weaker than that of Oxali (Fig. 3C-3D). Both Oxali and HDACi induced p300 nuclear translocation in murine PCa cells (Fig. 3D) and human PCa organoids (SI Appendix, Fig. S5A). ChIP experiments showed that Oxali induced p300 and RELA/p65 recruitment to the *Nlrc5* and *Ifngr2* promoters and p300 recruitment to the *Tap1, Psmb8/9* promoters (Fig. 3E-3F). These promoter regions also exhibited increased H3K14 and K27 acetylation after Oxali treatment (Fig. 3G). Oxali-induced H3K14 acetylation at nuclear foci, similar to those revealed by p300 antibody staining, was also observed by IF analysis (SI Appendix, Fig. S5B). Increased RELA/p65 K310 acetylation, which was attenuated after treatment by p300/CBP inhibitors, was confirmed by IB and IF analyses (SI Appendix, Fig. S5C-S5D). Using HA- or Myc-tagged *p300* and Flag-tagged *Stat1* expression vectors followed by immunoprecipitation (IP), we confirmed binding of p300 to endogenous RELA/p65 and transfected STAT1 (SI Appendix, Fig. S5E), an interaction that stimulates p300 acetyltransferase activity (36). To investigate the basis for p300 nuclear translocation, we examined induction of HLA-B–associated transcript 3 gene product, BAT3, which controls intracellular p300 distribution (37). IF analysis confirmed Oxali-induced nuclear translocation of both p300 and BAT3 (SI Appendix, Fig. S5F).

p300 and CBP control MHC-I AgPPM expression and neoantigen amounts
We generated cell lines deficient in p300 or CBP (Fig. 4A and SI Appendix, Fig. S6B). p300Δ-Myc-CaP cells expressed CBP and upregulated its expression upon Oxali treatment, and CBPΔ-Myc-CaP cells behaved similarly. Notably, p300Δ-Myc-CaP cell viability did not differ from that of parental cells and neither p300 nor CBP ablation reduced total or Oxali-induced total RELA/p65 protein or *Nfkbia* mRNA (Fig. 4A and SI Appendix, Fig. S6A-S6C). However, *Tap1*, *Psmb9*, *Nlrc5*, *Infgr2* and *Ifna* mRNA inductions were attenuated by both p300 or CBP ablations, whereas *Irf1* and *Erap1* mRNA inductions were only reduced in p300Δ-Myc-CaP cells (Fig. 4B-4F and SI Appendix, Fig. S6D-S6E). Both the p300 and CBP deficiencies attenuated induction of *Sec22b* mRNA (Fig. 4G), coding for a vesicle-trafficking protein that regulates phagosomal maturation and antigen crosspresentation (38). Consequently, both deficiencies hampered Oxali- and Mito-induced H-2Kq mRNA and surface expression (Fig. 4H and SI Appendix, Fig. S6F-S6G). A p300/CBP inhibitor also attenuated H-2Kq protein, and *Psmb9* and *Tap1* mRNA inductions (SI Appendix, Fig. S6H-S5I). Conversely, treatment of Myc-CaP cells with non-lethal

doses of the HDACi panobinostat (LBH589) induced *Nlrc5*, *Psmb9,* and *Tap1* mRNAs and surface H-2Kq (SI Appendix, Fig. S6J-S6L).

To gather information about p300 and CBP in human cancer, we examined the TCGA data set and found significant correlations between *EP300* or *CBP* mRNAs and genes identified by our integrative RNA-seq and ATAC-seq analyses, including *RELA*, *STAT1*, *NFKB1*, *IFNGR2* and *NLRC5* (Fig. 5A-5B, and SI Appendix, Fig. S7A). Similar correlations were found between histone modifiers and genes involved in MHC-I AgPP and T cell inflammation, particularly in human liver cancer (SI Appendix, Fig. S7B-S7C). Cancers with *EP300/CBP* loss-of-function [LOF; deletion and/or copy number variants (CNV) loss] showed lower *ERAP1* and *IFNGR1* or HLA-A expression (Fig. 5B). Curiously, EP300 and CBP were described both as oncogenes and oncosuppressors (39, 40). Phenotypes associated with heterozygous alterations were described in B cell lymphoma and Rubinstein-Taybi syndrome-1 (41, 42), suggesting dosage-dependent EP300/CBP function. We found that multiple CNVs, both gains and losses, affected *EP300* and *CBP* in HCC (LIHC) and PCa (PRAD) (Fig. 5A, and SI Appendix, Table S1) and correlated with their expression (SI Appendix, Fig. S7D). Interestingly, increased *EP300* and *CBP* copy numbers correlated with improved LIHC patient survival (Fig. 5C). We also analyzed CNVs of genes involved in MHC-I AgPPM and IFNγ signaling (Fig. 5D-5E, SI Appendix, Fig. S8A and Table S1). We found that LIHC and PRAD patients with gains in MHC-I AgPPM genes showed more frequent *EP300/CBP* LOF (Fig. 5D-5E, SI Appendix, Table S1), suggesting a compensatory mechanism that allows cancers with elevated MHC-I AgPPM evade immune-recognition. We also analyzed the number and the fraction of neoantigens in different cancers (SI Appendix, Fig. S8B-S8C). Remarkably, cancers with *EP300/CBP* LOF showed higher neoantigen amount (Fig. 5F and SI Appendix, Fig. S8D), supporting the notion that tumor immunoediting shapes the neoantigen landscape (43, 44) and that EP300/CBP may be part of this process.

NF-κB signaling and MHC-I AgPPM induction
Electron microscopy (EM) suggested that Oxali-treated cells underwent nucleolar/ribosomal and mitochondrial stress indicated by the condensed appearance of both organelles (Fig. 6A-6B, and SI Appendix, Fig. S9A). However, the absence of nuclear or mitochondrial fragmentation confirmed that most of the stressed cells remained viable. Oxali-induced nucleolar/ribosomal stress (45, 46), which was confirmed by Mass Spectrometry (MS) and RNA-seq analyses (SI Appendix, Fig. S9B-S9E), can account for NF-κB activation (47). To determine NF-κB's role in the response to Oxali, we generated RELA/p65-deficient cell lines (SI Appendix, Fig. S10A-S10B). Consistent with the ChIP experiments shown above (Fig. 3E), RELA/p65 was needed for full induction of *Ifngr2, Tap1, Psmab9, Nlrc5 and Bat3* mRNAs by low dose Oxali (Fig. 6C-6E, and SI Appendix, Fig. S10C-S10D). RELA/p65 ablation strongly inhibited surface H-2Kq induction by Oxali but barely affected the response to IFNγ (Fig. 6F). BAT3 and p300 nuclear translocation was also attenuated in RELA-deficient cells (Fig. 6G, and SI Appendix, Fig. S10E). Treatment of Myc-CaP cells with IKKβ inhibitors also reduced H-2Kq surface expression (SI Appendix, Fig. S10F). We also generated IRF1, STING/cGAS, STAT1, IFNγR2 and VDAC1 deficient Myc-CaP cells (Fig. 6H and SI Appendix, Fig. S11A-S11E). VDAC1 (voltage dependent anion channel 1) was recently shown to be required for the cytoplasmic release of mitochondrial (mt) DNA (48), which is considerably elevated in Oxali-stressed cells (SI Appendix, Fig. S11E-S11F). Notably, VDAC-1 ablation strongly reduced *Rela*, *p300* and *Irf-1* mRNA induction by Oxali (SI Appendix, Fig. S11G). Ablation of VDAC1 and IRF1, but not STAT1 or IFNγR2 abrogated Oxali-induced expression of surface H-2Kq and MHC-I AgPPM genes (Fig. 6H, and SI Appendix, Fig. S11H-S11K). Ablation of cGAS led to a small decrease in H-2Kq expression and no effect on induction of most AgPPM genes (Fig. 6H, and SI Appendix, Fig. S11H-S11K). Not surprisingly, STAT1, IRF-1 and IFNγR2 as well as VDAC1 and cGAS were required for H-2Kq surface expression in Myc-CaP cells treated with IFNγ alone or IFNγ + Oxali (SI Appendix, Fig. S11L). Oxali treatment also led to modest induction of PD-L1, a response that was enhanced by exogenous IFNγ

and was IRF1-dependent (SI Appendix, Fig. S11M), which has previously been shown to contribute to efficacy of ICIT (49). PD-L1 induction was not affected by TAP1 ablation, which completely prevented H-2Kq surface expression.

Chemotherapy induced functional antigen presentation

To confirm that Oxali stimulates neoantigen presentation we used MS to determine the peptidomes of H-2Kb and H-2Db molecules isolated from MC-38 cells after treatments, as described previously (50). Treatment with IFNγ + Oxali induced higher amounts (based on area under the curve) of H-2Kb-bound peptides relative to Oxali or IFNγ alone (SI Appendix, Fig. S12A). Although IFNγ led to higher amounts of H-2Db-bound peptides than Oxali in this particular cell line, chosen for its high MHC-I expression, some peptides were more efficiently presented after IFNγ + Oxali treatment.

The T cell activating ability of the Oxali-induced MHC-I bound peptides was confirmed using TRC2 PCa cells expressing high-, medium-, and low-affinity ovalbumin (Ova) variants. Oxali treatment stimulated H-2Kb presentation of the SIINFEKL epitope, especially in TRC2-N4 cells made to express the high-affinity (WT) variant (SI Appendix, Fig. S12B). When incubated with OT-I CD8$^+$ T cells, whose T-cell receptor (TCR) is SIINFEKL-specific, Oxali treated TRC2-N4 cells were more readily killed by activated OT-I T cells (SI Appendix, Fig. S12C-S12D). OT-I T cells enhanced presentation of the WT SIINFEKL epitope by TRC2-N4 cells in the absence of Oxali but had no effect on cells expressing the medium (TRC2-G4)- or low (TRC2-E1)-affinity variants. These results are consistent with a previous publication showing that only the high-affinity SIINFEKL epitope induces IFNγ secretion by OT-I cells (51), and further establish that the effect of Oxali is mechanistically distinct from that of IFNγ and dependent on neoantigen affinity and TCR activation.

We examined mouse and human cancer cell lines that differ in basal MHC-I expression. As described above, cells with high basal MHC-I such as MC-38 and B16 melanoma showed a weak response to platinoids alone but that response, including *Nlrc5* mRNA and surface MHC-I, was augmented by IFNγ (SI Appendix, Fig. S13A-S13B). In other cancer cells, e.g. the mouse melanoma YUMM cell lines, we observed a considerable variation in the response (SI Appendix, Fig. S13C). Strong Oxali-induced MHC-I surface expression was detected in human PC3 PCa cell line, PCSD1 cells, a 3D organoid culture from a Patient-Derived Xenograft (PDX) of bone metastatic PCa, certain primary melanoma cells and MIA PaCa-2 cells, representing ICIT-refractory PDAC (SI Appendix, Fig. S13D-S13G).

Activation of p300/CBP and NF-κB is needed for Oxali + anti-PD-L1 synergy

We sorted tumor-infiltrating CD8$^+$ T cells (TI-CD8$^+$) from subcutaneous (s.c.) Myc-CaP tumors, treated with either Oxali, anti-PD-L1, Oxali + anti-PD-L1 (combo) or left untreated (Ctrl) and performed scRNA-seq (Fig. 7A, SI Appendix, Fig. S14A). Several clusters of TI-CD8$^+$ cells with distinguishable gene expression and cluster specific pathway enrichment patterns were detected (Fig. 7B, and SI Appendix, Fig. S14B-S14H). Notably, elevated *Gzmb*, *Gzam*, *Prf1*, *Tbx21(Tbet)* mRNAs were detected in TI-CD8$^+$ from combo-treated mice (SI Appendix, Fig. S14E). Only combo therapy was associated with a significantly higher Teff signature (Fig. 7C and SI Appendix, Fig. S14F-S14H).

Next, we examined the involvement p300, CBP, IFNγR2 and NF-κB/RelA in Oxali-enhanced and CTL-mediated rejection of Myc-CaP tumors (Fig. 7A). The synergistic inhibition of tumor growth by Oxali + anti-PD-L1 was completely abrogated by p300 and CBP ablation in Myc-CaP cells (Fig. 7D-7G, and SI Appendix, Fig. S15A). IFNγR2 ablation also abolished the response to Oxali + anti-PD-L1 (Fig. 7G-7H and SI Appendix, Fig. S15B). As found in vitro, low-dose Oxali induced expression of *Ifngr2*, *Tap1*, *Psmb9*, and *Nlrc5* mRNA in Myc-CaP tumors (Fig. 7I-7K and SI Appendix, Fig. S15C). PD-L1 blockade did not affect *Ifngr2* mRNA expression, although it potentiated *Tap1*, *Psmb9*, and *Nlrc5* mRNA induction by Oxali, probably through IFNγ secretion by reinvigorated CTLs (Fig. 7I-7K and SI Appendix, Fig. S15C).

Indeed, IFNγR2 ablation had little effect on the response to Oxali alone while abrogating the response to Oxali + anti-PD-L1. *Tap1, Psmb9,* and *Nlrc5* induction by Oxali or Oxali + anti-PD-L1 was abrogated after p300 ablation (Fig. 7I-7K). IFNγR2 and p300 ablation also attenuated therapy-induced MHC-I (H-2Kq and H-2Dd) surface expression on CD45⁻ cancer cells (Fig. 7L, and SI Appendix, Fig. S15D-S15E), but had no effect on PD-L1 expression (SI Appendix, Fig. S15F). IFNγR2 and p300 ablations also had no effect on H-2Kq expression by tumor-infiltrating CD11c⁺ dendritic cells (SI Appendix, Fig. S15G). In accordance with scRNA-seq data, the Oxali+anti-PD-L1 combo increased the percentage and/or total numbers of tumor-infiltrating CD8⁺ and CD4⁺ T cells, CD107⁺IFNγ⁺ CTLs, IFNγ⁺ CD8⁺ and TNF⁺IFNγ⁺ CD8⁺ T cells, CD8⁺CD44⁺ Teff cells and CD8⁺CD44⁺PD1⁺TIM-3⁺ T cells analyzed by flow cytometry (Fig. 7M-7N and SI Appendix, Fig. S16A, S16C-S16H). Similar results were obtained for splenic CD8⁺ T cells (SI Appendix, Fig. S16B, and S16I-S16K). Of note, p300 or IFNγR2 ablation had little effect on tumor infiltrating effector CD8⁺ T cells, whose numbers were similarly increased after Oxali + anti-PD-L1 treatment in p300- or IFNγR2-expressing and non-expressing tumors (Fig. 7M-7N, and SI Appendix, Fig. S16C-S16H). By contrast, the Oxali + anti-PD-L1 combo decreased the fractions of each tumor occupied by CD45⁻ "cancer" cells, an effect that was most pronounced in WT tumors relative to p300 or IFNγR2 ablated tumors (Fig. 7O). NF-κB/RelA ablation also abolished the response to Oxali + anti-PD-L1 (Fig. 8A), consistent with its requirement for MHC-I and IFNγR2 induction (Fig. 8B, and SI Appendix, Fig. S17A), Thus, Oxali-induced upregulation of MHC-I AgPPM genes in malignant cells is important for the final recognition and killing stage of the cancer-immunity cycle (25) but has no role in ICIT-induced CTL reinvigoration.

Of note, ICD-mediated T cell priming proceeded normally in the absence of p300. FVB mice were immunized with Oxali-killed p300-proficient or -deficient Myc-CaP cells and challenged one week later with vital p300-proficient or -deficient Myc-CaP cells (Fig. 8C and SI Appendix, Fig. S17B). Myc-CaP cells grew significantly slower in FVB mice immunized with either p300-proficient or -deficient Myc-CaP cells compared to non-vaccinated mice, indicating that p300 has no effect on ICD-mediated T cell priming.

Discussion

ICIT-induced tumor rejection depends on activation of the cancer-immunity cycle, initiated by priming of tumor-directed T cells and terminated by killing of the targeted cancer cells by effector CTLs (16, 25). T cell priming can be enhanced by certain chemotherapeutic drugs capable of inducing ICD (14) and ICIT (4, 27). Nonetheless, even highly effective T cell priming and ICIT do not ensure successful CTL-mediated tumor killing, which requires MHC-I mediated presentation of tumor specific antigens (2, 14, 17, 52). Many cancers, especially PCa (SI Appendix, Fig. S7E) (53), evade immune elimination by downregulating MHC-I molecules or essential AgPPM components (11). Here we show that two different chemotherapeutic drugs, oxaliplatin (Oxali) and mitoxantrone (Mito) used at rather low concentrations, enhance CTL-mediated cancer cell recognition and killing through transcriptional induction of MHC-I AgPP (Schematic summary, SI Appendix, Fig. S17C). Although induction of MHC-I antigen presentation by chemotherapy and radiotherapy was described (54–56), the underlying mechanisms were only partly explored and attributed to type I IFN signaling. However, recent reports showing that sustained type I IFN signaling contributes to anti-PD-(L)1 resistance (57, 58) cast doubt on the role played by type I IFN in chemotherapy or radiotherapy induced immune stimulation. Our results show that Oxali renders low MHC-I expressing PCa cells responsive to anti-PD-1 therapy through transcriptional activation of the MHC-I AgPPM by NF-κB and p300/CBP, but not via the IFN-responsive TF STAT1. Ablation of p300 (or CBP) or NF-κB/RelA abolished the ability of low dose Oxali to synergize with anti-PD-L1 and induce rejection of Myc-CaP tumors. Consistent with their direct involvement in transcriptional activation of MHC-I AgPPM genes, ablation of p300 or RelA abrogated *Tap1*, *Psmb9* and *Nlrc5* induction in Myc-CaP tumors, but had no effect on tumor infiltration by effector CD8⁺ cells. Tumor infiltrating CTLs, however, were strongly increased after anti-PD-L1 + Oxali treatment as indicated by scRNA-seq and flow

cytometry. In contrast, ablation of p300 had no effect on the ability of Oxali-killed Myc-CaP cells to prime anti-tumor immunity, as indicated by vaccination experiments.

Oxali treatment triggers nucleolar/ribosomal stress (45, 46), possibly through its preferential interaction with rDNA or inhibition of rRNA synthesis, which represents almost half of the human genome (59). By virtue of its highly repetitive nature, rRNA integrity and expression are also sensitive to loss of topoisomerase II activity (60), a sequelae of Mito treatment. EM analysis of Oxali treated Myc-CaP cells confirmed altered nucleolar morphology, consistent with nucleolar stress, which can trigger NF-κB activation (47). By inducing *Bat3* transcription, RelA/NF-κB supports p300/CBP nuclear translocation, further increasing its own activity and stimulating histone acetylation. Oxali treatment can also enhance NF-κB activity via mitochondrial stress, whose presence in Myc-CaP cells is suggested by increased mitochondrial density and appearance of fragmented mtDNA in the cytosol. Ablation of VDAC1, through which mtDNA exits the mitochondrion (48), reduced *p300* and *Rela* mRNA expression and abrogated induction of NLRC5 and different MHC-I AgPPM components. NF-κB is also needed for induction of IFNγR2. Although IFNγR2 ablation had no effect on Oxali-induced MHC-I surface expression in cultured cells, it abrogated the rejection of Myc-CaP tumors and inhibited induction of MHC-I AgPPM genes in mice treated with anti-PD-L1 + Oxali. We postulate that IFNγR2 induction in Myc-CaP cells makes them more responsive to IFNγ secreted by tumor infiltrating CTL.

Neither Oxali nor Mito were developed as immunostimulatory drugs. It is therefore understandable that their immunogenic activity depends on multiple signaling pathways that are activated on induction of sub-lethal DNA damage and nucleolar and mitochondrial stress. Given the number of different signaling pathways activated by Oxali or Mito, it is rather surprising that ablation of either p300/CBP or RelA results in almost complete inhibition of the drug-induced immunogenic response. These findings parallel the cardinal importance of p300/CBP and NF-κB in activation of the MHC-I AgPP system. p300 plays a key role in assembly of the NLRC5 transcriptional activation complex and NF-κB recruitment to MHC-I genes (61). Notably, downregulation of NLRC5 has been observed in multiple cancer types, resulting in evasion of immune elimination (12). Conversely, we found that cancers with NLRC5 gain were more likely to undergo *CPB/EP300* loss. We also found that *EP300* and *CBP* LOF mutations and CNVs are rather common in certain types of cancer, and that their presence correlates with reduced MHC-I AgPPM expression. These genetic alterations seem more common than HLA loss mutations. Moreover, HCC and PCa with gain of *HLA*, *PSMB* and *TAP* genes, possibly due to chromosome 6p amplification (62), show higher frequency of *CBP/EP300* loss, which may allow them to undergo immune evasion. Based on its loss in several types of cancer, *EP300* was suggested to behave as a tumor suppressor gene (63, 64). We suggest that *CBP/EP300* loss promotes tumor growth by enabling immune evasion. One way to restore recognition of tumors with monoallelic *EP300/CBP* loss is treatment with low-dose Oxali or Mito or more potent and specific EP300/CBP activators.

Material and Methods

Detailed information about the animal models, in-vivo and in-vitro studies, flow cytometry, qRT-PCR, Immunoblot analysis, bioinformatic analysis, statistics and materials is provided in SI Appendix Material and Methods and Table S2.

Data and Materials Availability
This study did not generate new unique materials. The Sequencing data are available in NCBI's Gene Expression Omnibus database. Mouse ATAC-seq (GSE126287) and RNA-seq (GSE126274). Single cell RNA-seq data from live CD8^{+}CD3^{+} tumor infiltrating cells are available under the GEO accession number GSE151611.

Acknowledgements

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Figure legends

Fig. 1. Chemotherapy induces MHC-I AgPPM genes**.** (A) Heatmap showing all DEGs identified in bulk RNAseq of Myc-CaP cells treated with IFNγ (0.2 or 2 ng/mL), Oxali (2 μM), or both (Combo) (left). Venn diagram shows overlapping DEGs between IFNγ (2 ng/mL), Oxali and Combo treatment groups relative to Ctrl (middle) and Heatmap shows all DARs identified in bulk ATAC-seq of Myc-CaP cells treated as indicated relative to control (right). (B) GSEA was applied to expression profiles specific to each treatment group relative to Ctrl. Top 30 significantly enriched pathways for each respective comparison are shown. Some pathways were considered both IFNγ and Oxali driven (purple), while others were specific to either IFNγ (blue) or Oxali (red). (C) Functional enrichment was applied to genes classified with additive response to combination therapy. The top 20 enriched REACTOME pathways are shown.

Fig. 2. Transcriptional regulators of Oxali-induced MHC-I AgPPM genes**.** (A) RNAs from Myc-CaP cells incubated as indicated with IFNγ, Mito, Oxali, Carbo, or Cispl for 48 h were analyzed by qRT-PCR using *Nlrc5* primers*.* (B-C) Candidate genomic loci for *Nlrc5* (B) and *Ifngr2* (C), showing library-size normalized read pair pileup profiles determined by ATAC-seq across samples. Expression of respective genes determined by RNA-seq is also shown. (D) RNAs from Myc-CaP cells incubated as indicated were analyzed by qRT-PCR using *Ifngr2* primers. (E) Myc-CaP cells incubated with Oxali for 12 h were stained with β2M antibody (green) and phalloidin (red) and counterstained with DAPI. Magnification bar: 20 μm. (F) Myc-CaP cells treated as indicated were analyzed for surface MHC-I (H-2Kq) expression by flow cytometry. One-way ANOVA analysis (***) and multiple comparison confirmed the results. (G) Myc-CaP cells were incubated as indicated and lysed. LMP7 (PSMB8) immunoproteasome activity was measured using a fluorogenic LMP7-specific substrate peptide Ac-ANW-AMC. (H) Candidate genomic locus for *Nfkb1* showing read density profiles determined by ATAC-seq across samples. (I) Transcriptional regulator inference analysis (LISA) was applied to DEGs identified in comparisons of IFNγ (2 ng/mL), Oxali (2 μM), and both (Combo) treated cells relative to control, as well as to genes classified with additive response (top 500 upregulated, downregulated DEGs). The top 20 enriched regulators of upregulated (red) and downregulated (blue) DEGs are noted. (J) Transcriptional regulator binding sites enrichment analysis (GIGGLE) was applied to DARs identified in comparisons of IFNγ, Oxali, and Combo treated cells relative to control, as well as to regions classified with additive response. The top 20 enriched regulators of opened (red) and closed (blue) DARs are noted. (K) 200 common genes were identified by comparing TF found by DEGs and DARs analysis. (L) GSEA was applied to expression profiles determined in Oxali treated cells relative to control. The literature-curated known regulatory elements from ORegAnno database are shown. Candidate enrichment plots for representative pathways related to histone methylation (top) and acetylation (bottom) are shown. Two-sided t-test (means ± s.e.m), and Mann–Whitney test (median) were used to determine significance between two-groups. One-way ANOVA analysis and multiple comparison confirmed the results.*P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in (A, D, F, G), each experiment includes at least three biological replicates.

Fig. 3. Oxaliplatin and mitoxantrone stimulate HAT activity and nuclear localization. (A-B) Myc-CaP cells incubated with Oxali (2 or 4 μM) or the HDACi panobinostat (LBH589; 20 nM) for the

indicated times were lysed and analyzed for HAT activity using H3 as a substrate. (C) Nuclear extracts of Myc-CaP cells treated with Oxali, Mito and/or IFNγ were IB analyzed for p300, acetylated-CBP/p300, acetylated-RELA/p65 (lysine K310) and lamin B1 (loading control). (D) Myc-CaP cells treated as indicated for 12 h were stained with anti p300 (green) and phalloidin (red; actin cables). Nuclei were counter stained with DAPI (blue). Magnification bar: 20 μm. (E-G) Untreated and Oxali treated Myc-CaP cells were subjected to ChIP analysis with control IgG and antibodies to p65/RelA, p300 as indicated (E-F) or acetylated H3 (lysine K9, K14, and K27) (G). Precipitation of the indicated promoter regions was determined by PCR. Two-sided t-test (means ± s.e.m), and Mann–Whitney test (median) were used to determine significance. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in (A, E), each experiment includes at least three biological replicates.

Fig. 4. p300 and CBP control Oxali-induced MHC-I AgPPM genes. (A) Parental (shRNA-Ctrl) and *p300* or *CBP* silenced Myc-CaP cells were incubated with Oxali for 48 h. Nuclear extracts were IB analyzed for p300, CBP, p65/RELA and HDAC1 (loading control). (B-G) RNA expression in above cells was analyzed by qRT-PCR with the indicated primers. (H) Parental and gene edited Myc-CaP cells were incubated with Oxali and analyzed for surface MHC-I (H-2Kq) expression by flow cytometry. Two-sided t-test (means ± s.e.m), and Mann–Whitney test (median) were used to determine significance unless indicated otherwise. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in (B-H). Each experiment includes at least three biological replicates.

Fig. 5. p300/CBP control MHC-I AgPPM expression in human cancers. (A) *EP300* and *CBP* CNV losses and gains in LIHC (n = 369) and PRAD (n = 492). Key: dark blue (homozygous deletion), light blue (one copy loss), white (no CNV), pink (one copy gain), and red (high amplification). (B) Comparison of immune gene expression between LIHC *EP300/CBP* LOF and non-LOF groups. Non-viral patients are included for comparison of *CBP*, *EP300*, *ERAP1*, and *IFNGR1* expression. HLA-A expression was compared between *EP300/CBP* LOF and non-LOF HBV-infected groups. (C) Kaplan-Meier survival curves of LIHC patients with single gain (n = 34), no CNV (n = 165), and single loss (n = 92) events in *EP300/CBP*. P-values are based on log rank test. (D-E) Enrichment comparison of LIHC (D) and PRAD (E) with or without *EP300/CBP* LOF or no CNV versus CNV gain in the indicated gene groups: MHC-I AgPPM (HLA/PSMBs/TAPs), STAT1/IFNGR1/IFNGR2, β2M and NLRC5. Fisher's exact test was used to determine significance. (F) Landscape of high affinity neoantigens in four tumor types: PRAD (n = 492), LIHC (n = 356), LUSC (n = 473), and SKCM (n = 445). Groups were separated into *EP300/CBP* LOF (green dots) and non-LOF for neoantigen analysis. Mutations receiving a rank score < 2 and < 0.5 were considered binding and strong binding, respectively. P-values are based on Wilcoxon rank-sum test. Expression of neoantigens was determined from TCGA mRNA-seq reads. Two-sided t-test (means ± s.e.m), and Mann–Whitney test (median) were used to determine significance unless indicated otherwise. One-way ANOVA analysis and multiple comparison confirmed the results. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in (A-F). Each experiment includes at least three biological replicates.

Fig. 6. Role of NF-κB signaling in Oxali-induced MHC-I AgPPM expression. (A, B) Myc-CaP cells treated as indicated were fixed and examined by electron microscopy. Magnification bars are indicated in each image. White arrows indicate nucleolar stress. 10 representative images from each treatment group were analyzed and mitochondria/cytoplasm ratios were determined (B, right). (C-E) Parental (shRNA-Ctrl) or *Rela/p65*-silenced Myc-CaP cells were incubated with Oxali as indicated. RNAs were analyzed by qRT-PCR with the indicated primers. The results were confirmed using three different *Rela/p65* shRNAs. (F) Myc-CaP cells described in (C) were treated as indicated and analyzed for surface MHC-I (H-2Kq) expression. (G) Parental or *Rela/p65*-silenced Myc-CaP cells were treated as indicated and stained with mouse-anti p300 (green) and rabbit-anti BAT3 (red). Nuclei were counterstained with DAPI (blue) (n = 3). Magnification bar: 20 μm. (J) Parental and gene edited (Ctrl, CRISPR/Cas9) or shRNA silenced Myc-CaP cells were treated as indicated and analyzed for surface MHC-I (H-2Kq) expression. (B-F, H) Two-sided t-test (means ± s.e.m), and Mann–Whitney test (median) were used to determine significance. One-way ANOVA and multiple comparisons were used to confirm significance. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant. Specific n values are shown in (B-F). Each experiment includes at least three biological replicates.
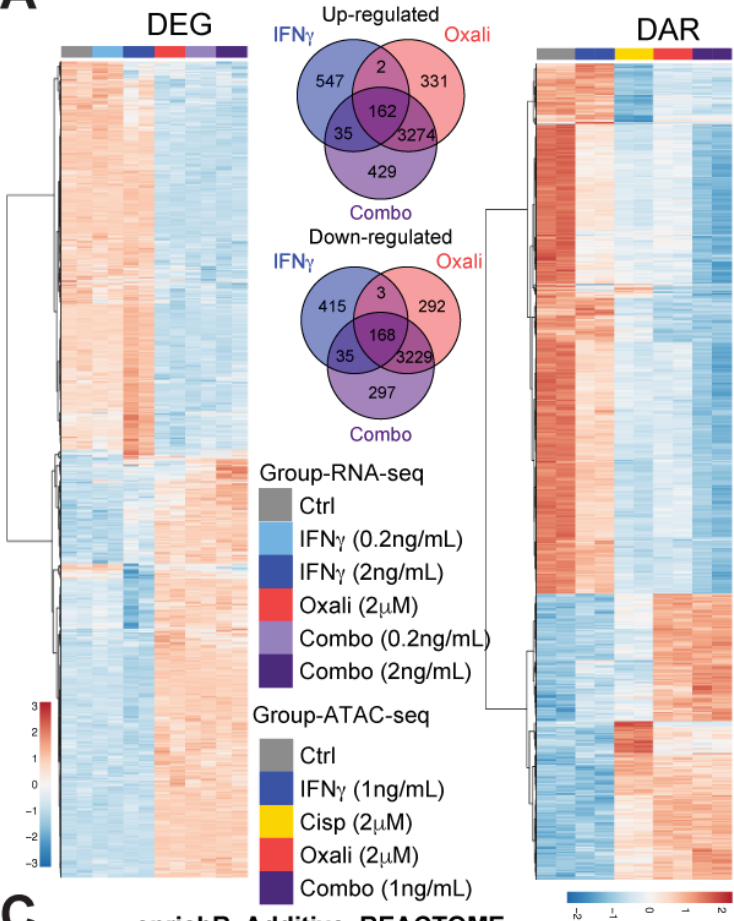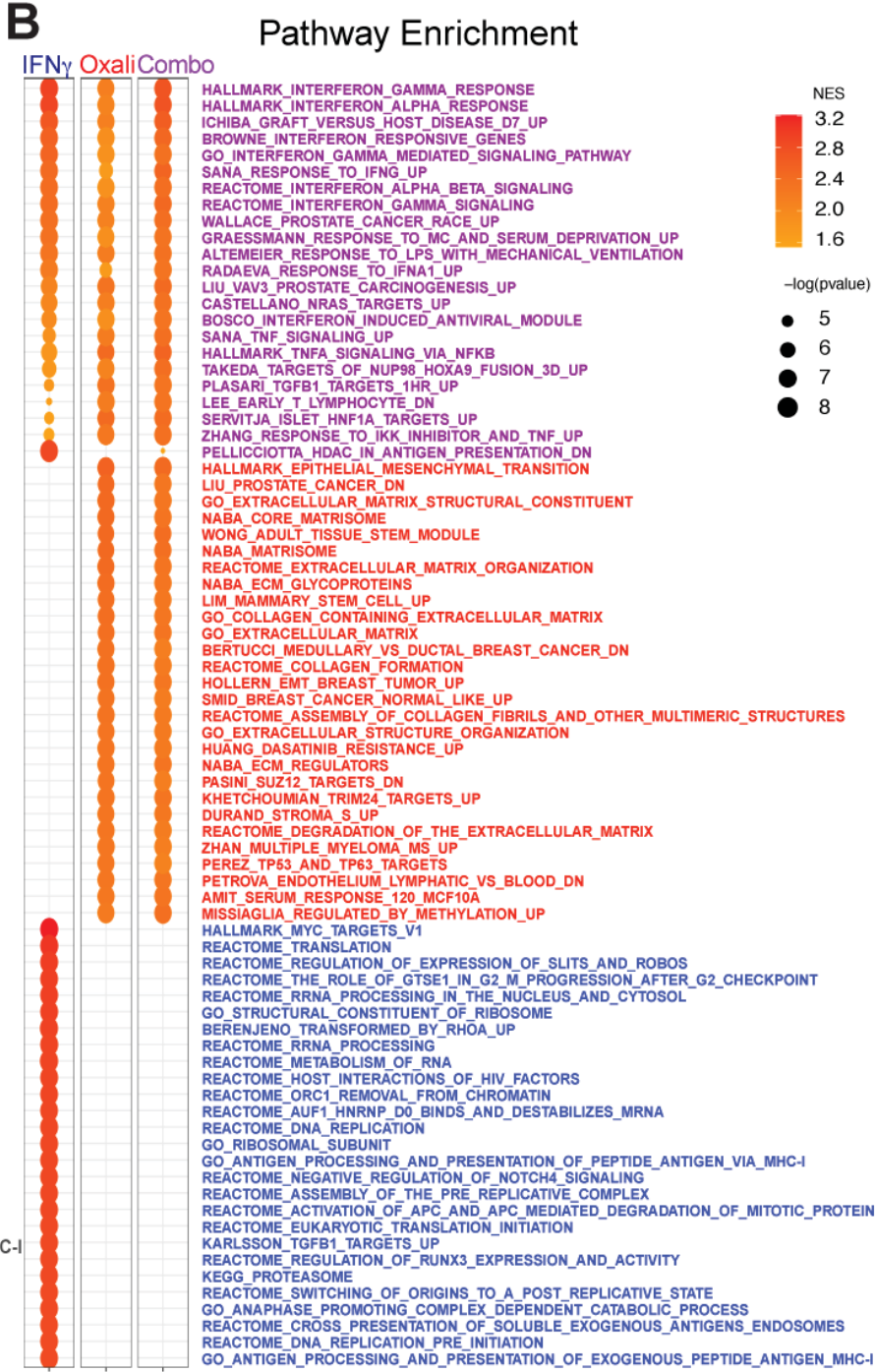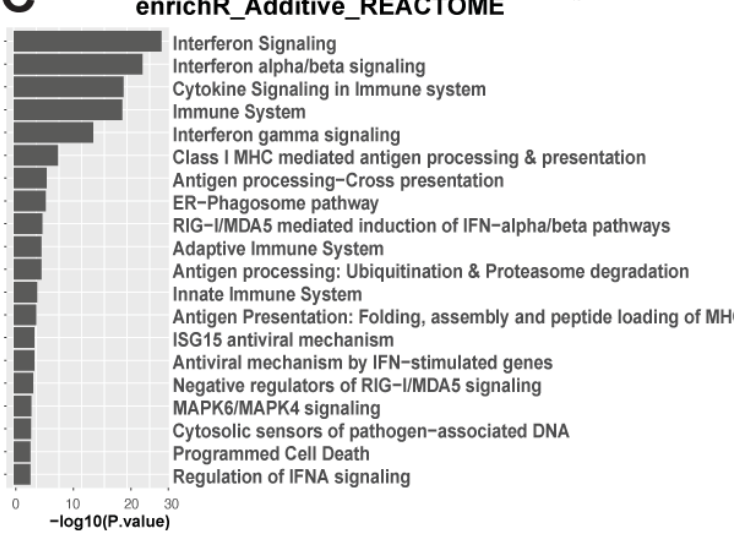
Fig. 7. Oxali + anti-PD-L1 synergy depends on p300/CBP and IFNγR2 expression. (A) Schematic description of in vivo experiments. Mice bearing s.c. Myc-CaP tumors were allocated into 4 treatment groups: (1) Control, (2) Oxali (6 mg/kg; weekly), (3) anti-PD-L1 (10 mg/kg; weekly), and (4) Oxali + anti-PD-L1 (weekly). After 4 treatment cycles, during which tumor size was measured,the mice were euthanized and analyzed. (B) UMAP representation of total T cell populations profiled by scRNAseq. Eleven distinct clusters were identified (Figure S14B-S14C). Proportional contributions of each cluster to sample-specific T cell populations is shown. Total cell numbers in each cluster are noted in Figure S14D. (C) Enrichment plots for candidate pathways defining CD8$^+$ Teff over Tmem and Tnaïve cells. Enrichment plots for the comparison between control and combo treatment is shown (other comparisons are shown in S14H). (D-F, H) Mice bearing s.c. Myc-CaP tumors generated by control (D), *p300*-silenced (E-F), and *Ifngr2* ablated (Clone 1) (H) cells were treated as described in (A). Specific *n* values are shown in each panel. Transient Cas9 expression and stable shRNA transfectants were used to avoid immune responses. Each dot is a treatment group, mean ± s.e.m. (G) Tumor weights for the indicated experimental groups (n = 3-8). (D-H) Two-sided t-test (means ± s.e.m) and two-way ANOVA were used to determine significance. (I-K) Total tumor RNA was analyzed by qRT-PCR for expression of the indicated genes. (L-N) Single cell tumor suspensions were analyzed for H-2Kq (L) expression on CD45$^-$ cells, total number of TI-CD8$^+$ (M) and effector CD8$^+$IFNγ$^+$CD107$^+$ T cell subsets (N). (O) Solid tumor composition (% cancer cells) was determined by gating on CD45$^-$ cells. (G, I-O) Each dot represents a mouse. Mann–Whitney test (median) was used to determine significance. One-way ANOVA and multiple comparisons were used to confirm significance. Specific *n* values are shown in D-O.

Fig. 8. Oxali-enhanced immune rejection requires NF-κB signaling. (A) Mice bearing s.c. Myc-CaP tumors generated by control and *RelA*-silenced cells were treated and analyzed as described in Figure 7A. Each dot represents a treatment group mean ± s.e.m. (B) Single tumor cell suspensions were analyzed for H-2Kq (left) and H-2Dd (right) expression on CD45$^-$ cells. (C) Scheme of vaccination experiments (left). Two groups of mice were immunized with lysates of Oxali-killed shRNA-ctrl (MC^{wt}) or *p300*-silenced (MC-p300^Δ) Myc-CaP cells. After 7 days, mice were s.c. inoculated with live shRNA-ctrl (MC^{wt}) cells. Live shRNA-ctrl (MC^{wt}) cells were also implanted into non-immunized mice as a control. Tumor growth curves are shown (right).

(A-C) Two-sided t-test (means ± s.e.m) and two-way ANOVA were used to determine significance. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific *n* values are shown in (A-C).

A

DEG

Up-regulated

IFNγ   Oxali
547   2   331
35   162   3274
429
Combo

Down-regulated

IFNγ   Oxali
415   3   292
35   168   3229
297
Combo

Group-RNA-seq
Ctrl
IFNγ (0.2ng/mL)
IFNγ (2ng/mL)
Oxali (2μM)
Combo (0.2ng/mL)
Combo (2ng/mL)

Group-ATAC-seq
Ctrl
IFNγ (1ng/mL)
Cisp (2μM)
Oxali (2μM)
Combo (1ng/mL)

DAR

B   Pathway Enrichment

IFNγ   Oxali   Combo

NES
3.2
2.8
2.4
2.0
1.6

−log(pvalue)
5
6
7
8

HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_INTERFERON_ALPHA_RESPONSE
ICHIBA_GRAFT_VERSUS_HOST_DISEASE_D7_UP
BROWNE_INTERFERON_RESPONSIVE_GENES
GO_INTERFERON_GAMMA_MEDIATED_SIGNALING_PATHWAY
SANA_RESPONSE_TO_IFNG_UP
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING
REACTOME_INTERFERON_GAMMA_SIGNALING
WALLACE_PROSTATE_CANCER_RACE_UP
GRAESSMANN_RESPONSE_TO_MC_AND_SERUM_DEPRIVATION_UP
ALTEMEIER_RESPONSE_TO_LPS_WITH_MECHANICAL_VENTILATION
RADAEVA_RESPONSE_TO_IFNA1_UP
LIU_VAV3_PROSTATE_CARCINOGENESIS_UP
CASTELLANO_NRAS_TARGETS_UP
BOSCO_INTERFERON_INDUCED_ANTIVIRAL_MODULE
SANA_TNF_SIGNALING_UP
HALLMARK_TNFA_SIGNALING_VIA_NFKB
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_3D_UP
PLASARI_TGFB1_TARGETS_1HR_UP
LEE_EARLY_T_LYMPHOCYTE_DN
SERVITJA_ISLET_HNF1A_TARGETS_UP
ZHANG_RESPONSE_TO_IKK_INHIBITOR_AND_TNF_UP
PELLICCIOTTA_HDAC_IN_ANTIGEN_PRESENTATION_DN
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
LIU_PROSTATE_CANCER_DN
GO_EXTRACELLULAR_MATRIX_STRUCTURAL_CONSTITUENT
NABA_CORE_MATRISOME
WONG_ADULT_TISSUE_STEM_MODULE
NABA_MATRISOME
REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION
NABA_ECM_GLYCOPROTEINS
LIM_MAMMARY_STEM_CELL_UP
GO_COLLAGEN_CONTAINING_EXTRACELLULAR_MATRIX
GO_EXTRACELLULAR_MATRIX
BERTUCCI_MEDULLARY_VS_DUCTAL_BREAST_CANCER_DN
REACTOME_COLLAGEN_FORMATION
HOLLERN_EMT_BREAST_TUMOR_UP
SMID_BREAST_CANCER_NORMAL_LIKE_UP
REACTOME_ASSEMBLY_OF_COLLAGEN_FIBRILS_AND_OTHER_MULTIMERIC_STRUCTURES
GO_EXTRACELLULAR_STRUCTURE_ORGANIZATION
HUANG_DASATINIB_RESISTANCE_UP
NABA_ECM_REGULATORS
PASINI_SUZ12_TARGETS_DN
KHETCHOUMIAN_TRIM24_TARGETS_UP
DURAND_STROMA_S_UP
REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX
ZHAN_MULTIPLE_MYELOMA_MS_UP
PEREZ_TP53_AND_TP63_TARGETS
PETROVA_ENDOTHELIUM_LYMPHATIC_VS_BLOOD_DN
AMIT_SERUM_RESPONSE_120_MCF10A
MISSIAGLIA_REGULATED_BY_METHYLATION_UP
HALLMARK_MYC_TARGETS_V1
REACTOME_TRANSLATION
REACTOME_REGULATION_OF_EXPRESSION_OF_SLITS_AND_ROBOS
REACTOME_THE_ROLE_OF_GTSE1_IN_G2_M_PROGRESSION_AFTER_G2_CHECKPOINT
REACTOME_RRNA_PROCESSING_IN_THE_NUCLEUS_AND_CYTOSOL
GO_STRUCTURAL_CONSTITUENT_OF_RIBOSOME
BERENJENO_TRANSFORMED_BY_RHOA_UP
REACTOME_RRNA_PROCESSING
REACTOME_METABOLISM_OF_RNA
REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN
REACTOME_AUF1_HNRNP_D0_BINDS_AND_DESTABILIZES_MRNA
REACTOME_DNA_REPLICATION
GO_RIBOSOMAL_SUBUNIT
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_PEPTIDE_ANTIGEN_VIA_MHC-I
REACTOME_NEGATIVE_REGULATION_OF_NOTCH4_SIGNALING
REACTOME_ASSEMBLY_OF_THE_PRE_REPLICATIVE_COMPLEX
REACTOME_ACTIVATION_OF_APC_AND_APC_MEDIATED_DEGRADATION_OF_MITOTIC_PROTEINS
REACTOME_EUKARYOTIC_TRANSLATION_INITIATION
KARLSSON_TGFB1_TARGETS_UP
REACTOME_REGULATION_OF_RUNX3_EXPRESSION_AND_ACTIVITY
KEGG_PROTEASOME
REACTOME_SWITCHING_OF_ORIGINS_TO_A_POST_REPLICATIVE_STATE
GO_ANAPHASE_PROMOTING_COMPLEX_DEPENDENT_CATABOLIC_PROCESS
REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSOMES
REACTOME_DNA_REPLICATION_PRE_INITIATION
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_EXOGENOUS_PEPTIDE_ANTIGEN_MHC-I

C   enrichR_Additive_REACTOME

Interferon Signaling
Interferon alpha/beta signaling
Cytokine Signaling in Immune system
Immune System
Interferon gamma signaling
Class I MHC mediated antigen processing & presentation
Antigen processing-Cross presentation
ER-Phagosome pathway
RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways
Adaptive Immune System
Antigen processing: Ubiquitination & Proteasome degradation
Innate Immune System
Antigen Presentation: Folding, assembly and peptide loading of MHC-I
ISG15 antiviral mechanism
Antiviral mechanism by IFN-stimulated genes
Negative regulators of RIG-I/MDA5 signaling
MAPK6/MAPK4 signaling
Cytosolic sensors of pathogen-associated DNA
Programmed Cell Death
Regulation of IFNA signaling

−log10(P.value)

A

HAT Specific Activity (ng/hr/mg)

Myc-CaP	1hr	3hr	6hr	12hr	24hr
HDACi (20nM)	- - - +	- - - +	- - - +	- - - +	- - - +
Oxali (μM)	- 2 4 -	- 2 4 -	- 2 4 -	- 2 4 -	- 2 4 -
n=	3 3 3 3	2 3 3 3	5 5 5 3	8 5 3 5	3 5 3 5

B

HATs Treatment Kinetics

Relative activity to control

Legend: ● 2μM Oxali ■ 4μM Oxali

x-axis: 1hr, 3hr, 6hr, 12hr, 24hr

C

							NE (kDa)
Oxali (2μM)	-	-	+	+	-	-	
Mito (2nM)	-	-	-	-	+	+	
IFNγ (2ng/mL)	-	+	-	+	-	+	
p300							300
Acetyl-CBP/p300							300
RELA/p65 (Acetyl Lys310)							65
Lamin B1							68

D

Columns: Ctrl | Oxali (2μM) | Oxali (4μM) | IFNγ (2ng/mL) | Oxali (2μM) + IFNγ 2ng/mL | HDACi (20nM)

Rows: p300 / DAPI / Phalloidin; p300; Phalloidin p300

Magnification Bar: 20μm

E

Nlrc5 Promoter

Nlrc5 promoter enrichment /18s Fold induction vs IgG

	Ctrl			Oxali (2μM)			Oxali (4μM)		
48hr IgG	+	-	-	+	-	-	+	-	-
anti-p65	-	+	-	-	+	-	-	+	-
anti-p300	-	-	+	-	-	+	-	-	+
n=	3	3	3	3	3	3	3	3	3

Ifngr2 Promoter

Ifngr2 promoter enrichment /18s Fold induction vs IgG

	Ctrl			Oxali (2μM)			Oxali (4μM)		
48hr IgG	+	-	-	+	-	-	+	-	-
anti-p65	-	+	-	-	+	-	-	+	-
anti-p300	-	-	+	-	-	+	-	-	+
n=	10	7	5	9	7	5	10	7	5

Legend: ■ Ctrl ■ Low Dose Oxali

F

Tap1 Promoter

Tap1 promoter enrichment /18s Fold induction vs IgG

48hr IgG	+	-	+	-
anti-p300	-	+	-	+

Psmb8/9 Promoter

Psmb8/9 promoter enrichment /18s Fold induction vs IgG

6hr IgG	+	-	+	-
anti-p300	-	+	-	+

G

Nlrc5 Promoter

Histone AcH3/ H3 ratio — 6hr: K9, K14, K27

Ifngr2 Promoter

Histone AcH3/ H3 ratio — 6hr: K9, K14, K27

Tap1 Promoter

Histone AcH3/H3 ratio — 6hr: K9, K14, K27

Psmb8/9 Promoter

Histone AcH3/H3 ratio — 6hr: K9, K14, K27

A

Ctrl | Oxali (2μM) 24h | Oxali (2μM) 48h

Magnification Bar: 3μm

B

Ctrl
Oxali (2μM) 24h
Oxali (2μM) 48h

Mito/cyto (density)

Oxali (2μM)	–	+	+
	–	24h	48h
n=	10	10	10

***

C

Ifngr2 mRNA (fold change)

MC 48h	shRNA Ctrl	shRNA *Rela/p65*
Oxali (2μM)	– +	– +
n=	3 3	9 9

***

D

Psmb9 mRNA (fold change)

MC 48h	shRNA Ctrl	shRNA *Rela/p65*
Oxali (2μM)	– +	– +
n=	3 3	9 9

***

E

Bat3 mRNA (fold change)

MC 48h	shRNA Ctrl	shRNA *Rela/p65*
Oxali (2μM)	– +	– +
n=	3 3	6 6

*** NS

F

H-2Kq (MFI-fold induction)

MC 48h	shRNA-Ctrl	shRNA-*Rela/p65*
IFNγ (2ng/mL)	– + – +	– + – +
Oxali (2μM)	– – + +	– – + +
n=	9 6 9 3	9 9 9 9

*** NS ***

G

MC 48h	MC-shRNA-Ctrl		MC-shRNA-*Rela*/p65-2	
	Ctrl	Oxali (2μM)	Ctrl	Oxali (2μM)

DAPI
p300
BAT3

Magnification Bar: 20μm

H

H-2Kq (MFI-fold induction)

	Ctrl	CRISPR *Ifngr2*−/−	CRISPR *Stat1*−/−	CRISPR *cGAS*−/−	CRISPR *Irf1*−/−	shRNA *Vdac1*
Oxali (2μM)	– +	– +	– +	– +	– +	– +

*** NS NS *** *** *** *** * *** ***

A MC-shRNA *Rela*

□ Ctrl (n=4)
▽ aPD-L1 (n=3)
▲ Oxali (n=3)
● Oxali+aPD-L1 (n=4)

NS

Tumor volumes (mm³)

Days

B

CD45⁻ H-2Kq (fold change)

** NS NS NS

MC	shRNA Ctrl	shRNA *Rela*
aPD-L1	- +	- + - +
Oxali	- +	- - + +
n=	7 6	4 3 3 3

CD45⁻ H-2Dd (fold change)

** NS NS NS

MC	shRNA Ctrl	shRNA *Rela*
aPD-L1	- +	- + - +
Oxali	- +	- - + +
n=	7 6	4 3 3 3

C

no-immunization → n=5 viable shRNActrl

Immunization (d0)
Oxali n=5
Myc-CaP → d7 → n=5 viable shRNActrl

Oxali n=5
shRNA *p300* → d7 → n=5 viable shRNActrl

□ MC^WT (n=6)
△ MC^WTvac / MC^WT (n=5)
● MC-p300^Δvac / MC^WT (n=5)

*** *** NS

Tumor volumes (cm³)

Days