A kinome-wide CRISPR screen identifies CK1α as a novel target to overcome enzalutamide resistance of prostate cancer

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Abstract

Enzalutamide (ENZA), a second-generation androgen receptor antagonist, has significantly increased progression-free and overall survival of patients with metastatic prostate cancer (PCa). However, resistance remains a prominent obstacle in treatment, illustrating the urgent need to develop new approaches to increase ENZA efficacy. Utilizing a kinome-wide CRISPR-Cas9 knockout screen, we identified casein kinase 1 alpha (CK1α) as a novel therapeutic target to overcome ENZA resistance. Depletion or pharmacologic inhibition of CK1α significantly enhanced EZNA efficacy in ENZA-resistant cell lines and patient-derived xenografts. Mechanistically, CK1α phosphorylates the serine residue S1270 and modulates the protein abundance of ataxia-telangiectasia mutated (ATM), a primary initiator of DNA double-strand break (DSB)-response signaling, which is compromised in ENZA-resistant cells and patients. Inhibition of CK1α stabilizes ATM, resulting in the restoration of DSB-response signaling, and thus increases ENZA-induced cell death and growth arrest in an ATM-dependent manner. Our study details an innovative therapeutic approach for ENZA-resistant PCa and characterizes a novel perspective for the function of CK1α in the regulation of DNA damage response signaling.

Introduction

Prostate cancer (PCa) is the most common non-cutaneous malignancy among males, and is also the leading cause of cancer-related death in the world[1]. Androgen deprivation therapy is the first line of therapy for metastatic PCa, but most patients will eventually progress into castration-resistant PCa (CRPC). The five-year survival rate of men with metastatic CRPC is only 31%[2], highlighting the urgent need for development of novel therapies. Enzalutamide (ENZA), a potent second-generation androgen receptor (AR) antagonist, has been approved for metastatic CRPC therapy and has shown remarkable improvement in patient survival outcomes[3]. Importantly, a recent large scale randomized trial established that ENZA could delay the time to metastasis and thereby prolong overall survival of patients with non-metastatic CRPC[4]. Nevertheless, most patients ultimately progress to ENZA resistance, which has unreliable and limited treatment options. Therefore, it is critical to investigate new treatments for ENZA-resistant PCa.

Various studies have defined characterization of ENZA resistance, which are divided into two general categories: AR-dependent and AR-independent mechanisms. AR-dependent ENZA-resistant mechanisms include restoration of AR activity due to AR amplification, AR point mutations that convert ENZA from an antagonist to an agonist, and expression of AR splice variants that are constitutively active due to lack of the ligand-binding domain[5]. AR-independent ENZA-resistant mechanisms are defined as bypassing AR signaling via other hormone nuclear receptors, such as glucocorticoid receptor[6], or acquiring lineage plasticity characteristics by expressing neuroendocrine and stem cell associated genes through epigenetic mechanisms[7, 8]. However, few therapeutic targets have moved forward to clinical trial or treatment.

Clustered regularly interspaced short palindrome repeats associated protein 9 (CRISPR-Cas9) technology is an efficient approach for gene editing, whereby Cas9, an endonuclease, cuts DNA at a location specified by a guide RNA. Loss-of-function pooled library screens using CRISPR-Cas9 have displayed a powerful ability to identify genes related to drug response[9, 10]. By use of an unbiased kinome-wide CRISPR-Cas9 library screen, we discovered casein kinase 1 alpha (CK1α) was a top candidate whose knockout sensitized cells to ENZA treatment. CK1α participates in extensive regulatory roles in numerous cellular processes including cell metabolism and differentiation in development, autophagy, chromosome segregation, cell cycle, apoptosis, immune response and neurodegeneration[11]. Mutations and alterations of CK1α are detected in various tumor entities including acute myeloid leukemia (AML), lung, esophageal, urothelial, kidney, breast, pancreas and ovarian cancers[11–13]. Moreover, CK1α expression is associated with poorer overall survival in cancer patients[14]. Additionally, CK1α has also been reported to be implicated in acquired drug resistance to erlotinib in non-small cell lung cancer[15]. These findings suggest that CK1α is a conditionally essential oncogene and a potential target for cancer therapy. However, whether and how CK1α is involved in PCa development and ENZA treatment response is ambiguous.

Results

A kinome-wide CRISPR-Cas9 knockout screen identifies CK1α as a novel therapeutic target to overcome ENZA resistance.

Protein kinases regulate nearly all aspects of cell life and their alterations or mutations contribute to cancer initiation and progression. Consequently, kinases have been major targets for cancer therapy. So far, 76 kinase inhibitors have been approved for use in the clinic and many others are in clinical trials[16]. To identify potential kinases whose inhibition might overcome ENZA resistance in PCa treatment, we performed a pooled kinome-wide CRISPR-Cas9 knockout screen in ENZA-resistant PCa 22Rv1 cells using a lentiviral single guide RNA (sgRNA) library (Fig. 1a) that contained 3,052 unique sgRNAs targeting 763 human kinases.

First, we generated a stable cell line expressing Cas9 in 22Rv1 cells (22Rv1-Cas9) (Extended Data Fig. 1a) and then validated its efficacy of gene knockout by targeting AR-full length with two sgRNAs (Extended Data Fig. 1b). We also compared the response of 22Rv1-Cas9 and parental 22Rv1 cells (22Rv1-WT) to ENZA to ensure that expression of Cas9 did not change the sensitivity of cells to ENZA (Extended Data Fig. 1c). After transduction at a low multiplicity of infection (MOI = 0.3) followed by selection with puromycin, 1/3 of the infected cells (500 × coverage) were harvested as the baseline for sequencing and the rest of the cells were continuously cultured for additional two weeks to allow gene editing (Fig. 1a). Then cells were randomly split and treated with ENZA or DMSO for one an additional week. Next, the sgRNAs incorporated in the cells were amplified from genomic DNA and subjected to sequencing, followed by Model-based Analysis of Genome-wide CRISPR-Cas9 Knockout (MAGeCK)[17] (Fig. 1b). Consistent with previous CRISPR screening[17, 18], multiple essential and non-essential genes identified in previous screens were also identified in our analysis (Fig. 1c), validating our approach. Among the genes

we identified, *CSNK1A1* (coding CK1α) was the top one candidate whose knockout enhanced the efficacy of ENZA in 22Rv1 cells (Fig. 1c; Extended Data, Table 1). Of note, the top two and three candidates, *MTOR* and *PLK1*, respectively, have been reported to be involved in ENZA-resistance in previous studies[19, 20]. In addition, a high level of CK1α correlates with poor overall survival in PCa (Extended Data Fig. 1d).

Validation of CK1α as a novel target to treat ENZA-resistant PCa.

To validate CK1α as an effective target to overcome ENZA-resistance in PCa, we compared the cellular response to ENZA upon CK1α deletion. As indicated, CK1α knockout significantly decreased cell viability and colony formation of 22Rv1 cells upon ENZA treatment (Fig. 2a; Extended Data Fig. 2a). Similarly, knockdown of CK1α also dramatically enhanced the efficacy of ENZA treatment in two other ENZA-resistant PCa lines: C4-2R (Fig. 2b; Extended Data Fig. 2b) and MR49F (Fig. 2c; Extended Data Fig. 2c). In addition, consistent with these findings, ENZA treatment induced a significant increase of cleaved PARP (c-PARP), a marker of apoptosis, in 22Rv1 cells with CK1α knockout but not 22Rv1-WT cells in a dose-dependent manner (Fig. 6f). Finally, overexpression of CK1α attenuated ENZA-induced elevation of c-PARP in ENZA-sensitive PCa cells, C4-2 (Fig. 6e).

Next, we tested these observations in an *in vivo* setting. Accordingly, castrated nude mice carrying xenograft tumors derived from wild-type 22Rv1 or 22Rv1 with CK1α knockout were subjected to treatment with ENZA (Fig. 2d), followed by measurement of tumor growth. As expected, CK1α deletion increased the sensitivity of 22Rv1 xenografts to ENZA treatment compared with 22Rv1-WT, as indicated by limited tumor growth (Fig. 2e) and reduced tumor weight (Fig. 2f; Extended Data Figs. 2d, 2e). Furthermore, with respect to clinical relevance, inhibition of CK1α with A51, a novel compound targeting CK1α currently in a clinical trial (NCT04243785) to treat AML, significantly enhanced the efficacy of ENZA in an ENZA-resistant patient-derived xenograft (PDX) model, LuCaP 35CR[21], and improved survival of the mice without apparent toxicity (Figs. 2g-2i; Extended Data Fig. 2f). Collectively, these results demonstrate that CK1α is a promising target to treat ENZA-resistant PCa.

CK1α regulates double-strand break (DSB)-response signaling.

To dissect the underlying mechanisms of targeting CK1α to sensitize cancer cells to ENZA, we performed RNA sequencing of 22Rv1 cells upon CK1α knockout or overexpression. With the Gene Set Enrichment Analysis (GSEA), we found that 287 gene sets were significantly enriched in CK1α-overexpressing cells compared with the control, 253 (206 plus 47) pathways down-regulated (Fig. 3a) and 34 (30 plus 4) pathways up-regulated (Fig. 3b). In contrast, 140 gene sets were significant enriched in CK1α-knockout cells compared with the control, 63 (47 plus 16) up-regulated (Fig. 3a) and 77 (73 plus 4) down-regulated pathways (Fig. 3b). Further analysis indicated that 51 (47 plus 4, Figs. 3a, 3b; Extended Data Table 2) gene sets were significantly enriched in both CK1α-knockout and CK1α-overexpressing cells but with the opposite enriched trend. Interestingly, of these 51 pathways, nearly 80% (40/51) were related to DNA-damage response (DDR) (Fig. 3c; Extended Data Figs. 3a, 3b), and, in particular, DNA double-strand break (DSB) repair by homologous recombination (HR) (Figs. 3d, 3e), which includes a series of pathways that

monitor DNA integrity, and activate cell cycle checkpoints and DNA repair. Specifically, knockout of CK1α activated the DSB-response, as indicated by the hyperactivity of DSB signaling related gene sets, such as G2/M DNA damage checkpoint (Fig. 3f), and DNA DSB repair pathways (Fig. 3g; Extended Data Figs. 3c-3h), whereas CK1α overexpression inhibited DSB-response signaling, validated by downregulation of these pathways (Figs. 3h, 3i; Extended Data Figs. 3i-3n). In addition, we also confirmed these findings with The Cancer Genome Atlas (TCGA) dataset of PCa patients. As indicated, patients with a higher level of CK1α (Fig. 3j) exhibited lower activity of G2/M DNA damage checkpoint and DNA DSB repair pathways (Figs. 3k, 3l), indicating that the correlation of CK1α and DSB response was not just a phenomenon in cultured cells. Taken together, these results suggest that CK1α negatively regulates DSB-response signaling, a possible mechanism for ENZA resistance.

DSB signaling is involved in ENZA resistance.

PCa cells often harbor germline or somatic alterations in DDR genes, which are associated with disease outcome and treatment response[22]. Interestingly, when reviewing the results of our CRISPR screen, we discovered that the deletion of kinases (*CHEK2*, *ATM*, *ATMIN*, *HUS1*, *NEK9* and *TP53RK*) in DSB-response signaling rendered 22Rv1 cells more resistant to ENZA (Fig. 4a; Extended Data Fig. 4a, Table 1). To validate this observation, we selected the top two candidates, *ATM* (coding ATM) and *CHEK2* (coding CHK2), then devised knockout for these genes in 22Rv1 and C4-2 cells, respectively. As indicated, knockout of CHK2 and ATM immensely improved cell survival upon ENZA treatment in 22Rv1 (Figs. 4b, 4c; Extended Data Figs. 4b, 4c) and C4-2 cells (Figs. 4d, 4e; Extended Data Figs. 4d, 4e). In addition, improvement of the cell survival was more significant in ENZA-sensitive C4-2 cells compared to ENZA-resistant 22Rv1 cells.

Recently, genomic alteration of *TP53* (coding p53), a key effector of the DSB response, was reported to be associated with a shorter time on treatment with the AR signaling inhibitors (ARSIs) in clinical outcome[23]. Consistent with this study, we also observed that deletion of *TP53RK* (coding TP53RK), a key upstream kinase that phosphorylates and activates p53, could induce ENZA resistance (Fig. 4a; Extended Data Fig. 4a). To further confirm the role of DSB signaling in the ENZA response, we also examined the effect of p53 abundance in the ENZA response. As expected, knockout of p53 significantly induced resistance to ENZA in two ENZA-sensitive cell lines, LNCaP and C4-2 (Extended Data Figs. 4f-4i).

Additionally, our findings are supported by RNA-sequencing data from both cell lines and human patients (Figs. 4f-4i). We performed RNA sequencing with ENZA-sensitive C4-2 cells, compared to ENZA-resistant C4-2R cells. As indicated, GSEA analyses revealed that the DSB-related gene sets, DNA DSB repair response, and G2/M DNA damage checkpoint pathways, have lower activity in ENZA-resistant C4-2R cells compared to ENZA-sensitive C4-2 cells (Figs. 4f, 4g). Furthermore, Alumkal at el. recently published a study[7] that characterized the genomic landscape of patients with *de novo* resistance to ENZA. This provides the opportunity to test the association of DSB signaling and ENZA response with a clinical dataset. Consistent with our findings *in vitro*, DNA DSB repair and G2/M DNA damage checkpoint pathways were also inactivated in patients who showed non-response to ENZA compared with

responsive patients (Figs. 4h, 4i). This suggests that DSB signaling is compromised in ENZA non-responders. Moreover, the “CK1α negative signature”, generated by overlapping the genes down-regulated by CK1α overexpression and up-regulated by CK1α knockout in 22Rv1 cells (Fig. 4j; Extended Data, Table 3), was also negatively enriched in ENZA non-responders compared with responders (Fig. 4k).

ATM is involved in CK1α-associated regulation of DSB signaling.

Next, to uncover the underlying mechanism of CK1α in the regulation of DSB signaling, we examined the indicator of DSB response, γH2AX, and the regulator of the G2/M checkpoint, CHK2, upon manipulation of CK1α. Expectedly, DSB signaling was activated upon CK1α knockout, as indicated by increased phosphorylation of H2AX (γH2AX) and CHK2, but decreased upon CK1α overexpression (Fig. 5a). Interestingly, the abundance of ATM, the primary initiator of the DSB response, was increased upon CK1α knockout, but decreased upon CK1α overexpression (Fig. 5a). Consistent with this observation, CK1α knockdown (Figs. 5b, 5c) or inhibition with two different selective inhibitors of CK1α kinase, A51 or D4476 (Figs. 5d-5h), in 22Rv1 and C4-2 cells dramatically upregulated the protein level of ATM as well as the phosphorylation of H2AX and CHK2. Moreover, this result was supported by *in vivo* experiments, as A51 treatment caused significant accumulation of ATM protein and γH2AX in patient-derived xenografts (Figs. 6g, 6h).

Subsequently, we investigated whether CK1α regulates the level of ATM in human PCa. To address this issue, we examined CK1α and ATM expression in patient samples by performing immunohistochemistry (IHC) on a tissue microarray containing a cohort of primary PCa specimens. IHC staining was evaluated by measuring both staining intensity and percentage of positive cells. Representative IHC images display low or high staining of CK1α as well as the signal of ATM in the same case (Figs. 5i, 5j). Further analysis showed that ATM protein expression is inversely correlated with the level of CK1α among the patients (Fig. 5k). This finding was further supported by GSEA analysis of RNA sequencing datasets from the TCGA PCa patients and cell lines; patients with a higher level of CK1α (Fig. 5l) or cells with overexpressed CK1α (Fig. 5m) displayed lower activity of the ATM pathway.

To further solidify these findings, we determined whether ATM was involved in CK1α-associated regulation of DSB signaling. Accordingly, ATM was knocked out utilizing two sgRNAs in 22Rv1 and C4-2 cells, followed by manipulating CK1α activity with the inhibitors A51 and D4476. CK1α inhibition significantly increased ATM abundance, as well as phosphorylation of CHK2 and H2AX in wild-type cells, whereas deletion of ATM dramatically attenuated CK1α inhibition-induced phosphorylation of CHK2 and H2AX (Figs. 5n, 5o). This finding revealed that ATM does participate in CK1α-mediated regulation of the DSB response. Furthermore, it has been reported that DNA-PKcs also regulated DSB signaling by phosphorylating H2AX and CHK2. We thus depleted DNA-PKcs in 22Rv1 and C4-2 cells, followed by CK1α inhibition. As indicated, deletion of DNA-PKcs did not attenuate CK1α inhibition-induced DSB signaling (Extended Data Figs. 5a, 5b). Overall, these results indicate that CK1α regulates DSB signaling by modulating the abundance of ATM.

ATM initiated-DSB signaling is involved in the modulation of the ENZA response by CK1α.

As described above, ATM and DSB response-related signaling are correlated with the ENZA response (Fig. 4). A previous study[23], focusing on the genomic landscape of metastatic CRPC with clinical outcomes, showed no significant correlation between genomic alterations of ATM and the treatment probability on first line ARSIs, ENZA and abiraterone. In contrast, with the transcriptome dataset of this study, we found ATM mRNA level was significantly associated with the time on treatment with ARSIs (Fig. 6a). Patients with a higher level of ATM expression, had a significantly longer time to progress on ARSIs treatment compared to patients with a lower expression of ATM (Figs. 6b, 6c). This finding is further supported by Cox hazards ratio analysis, which showed a significant decreased hazards in patients with a higher level of ATM, compared with patients with a lower level of ATM (Extended Data Fig. 5c). Moreover, this notion is also supported by the dataset from the study of Alumkal et al.[7], which showed that the ATM pathway was inactivated in patients who were categorized as non-responsive to ENZA (Fig. 6d). Of note, during the preparation of this manuscript, another clinical study reported that patients with circulating tumor cells (CTCs) harboring genomic gains of ATM had an enhanced clinical benefit to ARSIs[24]. Overall, these findings indicate that either ATM level or activity could be a prognostic biomarker for the clinical outcome of ARSIs.

Since CK1α regulates DSB signaling by modulating ATM abundance (Fig. 5), we sought to determine whether ATM-initiated DSB response was involved in regulation of the ENZA response by CK1α. A previous study showed that ENZA could induce a BRCA-deficient state, “BRCAness”[25]. Consistent with this finding, in ENZA-sensitive C4-2 cells, ENZA treatment activated DSB signaling and induced cell apoptosis, indicated by phosphorylation of H2AX and CHK2, and increased c-PARP (Fig. 6e). Overexpression of CK1α reversed these phenotypes through reducing ATM abundance (Fig. 6e). Conversely, in ENZA-resistant 22Rv1 cells, CK1α knockout stabilized ATM protein and sensitized cells to ENZA treatment, indicated by increased DSB response and apoptosis (Fig. 6f). These observations were further validated *in vivo* via a PDX treatment regime. Mono-treatment with ENZA in the ENZA-resistant PDX group did not cause obvious apoptosis, but significant cell death was observed when ENZA was combined with CK1α inhibition, accompanied by elevated levels of ATM and γH2AX (Figs. 6g-6i; Extend Data Fig. 5d). Consistent with these findings, ATM knockout rendered cells resistant to the selective inhibitors of CK1α, A51 (Extended Data Fig. 5e) and D4476 (Extended Data Fig. 5f). Furthermore, ATM knockout significantly attenuated CK1α inhibition-enhanced ENZA efficacy in 22Rv1 cells (Figs. 6j, 6k). Collectively, these findings suggest that ATM and ATM-associated DSB response signaling are essential for modulation of the ENZA response by CK1α.

CK1α regulates ATM stability by phosphorylating ATM at S1270.

To gain mechanistic insight into how CK1α regulates ATM abundance, we first checked our RNA sequencing data. However, neither CK1α knockout nor CK1α overexpression significantly affected the level of ATM mRNA (Extended Data Figs. 6a, 6b), suggesting that CK1α does not regulate the transcription level of ATM. Ectopic expression of CK1α significantly reduced ATM abundance (Figs. 7a, 7b) and induced ATM poly-ubiquitination (Fig. 7c), and MG132 could reverse CK1α-induced ATM degradation (Fig. 7b). Collectively, these observations suggest that CK1α regulates ATM stability in an

ubiquitin-dependent manner. Consistent with this notion, the half-life of ATM protein was significantly shorter in CK1α-overexpressing cells compared with wild-type cells (Fig. 7d).

Because CK1α functions as a kinase, the idea of whether ATM is a substrate of CK1α was explored. To further support this hypothesis, we detected a physical interaction between ectopically expressed or endogenous CK1α and ATM proteins in HEK293T and 22Rv1 cells (Figs. 7e, 7f; Extended Data Figs. 6c, 6d). Furthermore, an *in vitro* kinase assay showed that CK1α directly phosphorylated ATM (Fig. 7g), on a site(s) located in Fragment 3 (amino acid 830–1290) (Fig. 7h). Next, two sites, S1270, identified by mass spectrometry (Fig. 7i), and S1281, predicted by Group-based Prediction System (version 5.0), were considered as potential CK1α phosphorylation sites. We further showed that mutation of S1270 to alanine (S1270A), but not S1281, largely diminished CK1α-mediated phosphorylation of ATM (Fig. 7j). Subsequently, this finding is verified by a specific antibody recognizing ATM when phosphorylated at S1270 (p-ATM S1270), which was validated by an *in vitro* kinase assay. As indicated (Fig. 7k), only the incubation of CK1α kinase rendered ATM Fragment 3 (ATM-F3) be recognized by anti-p-ATM S1270 antibody, whereas ATM-F3 itself, ATM-F3-S1270A, or ATM-F3-S1270A incubating with CK1α kinase was undetectable by the antibody. Depletion or pharmacologic inhibition of CK1α dramatically decreased the phosphorylation of ATM at S1270 in PCa 22Rv1 (Figs. 7l, 7m) and C4-2 (Figs. 7n, 7o) cells. Overexpression of CK1α induced ATM phosphorylation at S1270, and CK1a kinase inhibitor, A51, significantly abolished this induction (Fig. 7p). Lastly, mutation of S1270 to alanine rendered ATM resistant to CK1α-meditated poly-ubiquitination (Fig. 7r) and degradation (Fig. 7q), and prolonged the half-life of ATM protein upon CK1α overexpression (Fig. 7s).

Discussion

Recently, the mechanism of lenalidomide's high efficiency in pre-leukemic myelodysplastic syndrome (MDS) with deletion of chromosome 5q (del (5q)) was addressed by identification of CK1α as a novel lenalidomide-regulated $CRL4^{CRBN}$ substrate[13]. CSNK1A1 (coding CK1α) is located in the deleted region of del (5q) MDS and the resultant haploinsufficient expression of CK1α sensitizes cells to lenalidomide treatment, although this is limited to patients with one active allele of CK1a. Recent identification of A51, a selective CK1α inhibitor[26] provides opportunity to the full therapeutic potential of targeting CK1a in cancer therapy. In this study, we identify CK1α, whose expression is correlated with poor overall survival of PCa, as a novel therapeutic target to overcome ENZA-resistance with a kinome-scale CRISPR-Cas9 knockout screen (Extended Data Fig. 6e). Of note, A51 has been approved by the U.S. Food and Drug Administration in clinical trials for patients with AML. In addition, A51 has shown the ability to block super enhancer targets (CDK7/CDK9) and prevent transcription of the key oncogene MYC, another established target for PCa therapy[26, 27]. Therefore, our findings will have a strong translational potential and will be beneficial to patients suffering with ENZA-resistant CRPC.

CK1α is a well-established regulator of β-catenin protein stability. CK1α phosphorylates β-catenin at S45, which is subsequently phosphorylated by GSK3β, leading to its degradation[28, 29]. Thus, CK1α inhibition would likely increase β-catenin abundance and activate Wnt signaling. While increase of β-catenin has

been linked to gain of ENZA resistance[30]. This paradox has also been documented in colon cancer models. Stabilizing β-catenin, a key driver of colon cancer, by homologous deletion of CK1α does not induce tumorigenesis[31]. The underlying mechanism of this observation is that CK1α loss induced-activation of β-catenin and the Wnt pathway is distinguished from other models of Wnt hyperactivation, such as APC ablation. CK1α loss would induce p53-dependent growth arrest and apoptosis, which counteracts the pro-tumorigenic effects of Wnt hyperactivation[31, 32]. Thus, CK1α is a double-edged sword. We propose that cells resistant to ENZA maintain the abundance of CK1α at a certain level in order to balance the Wnt-β-catenin pathway and DSB-response signaling to survive under ENZA treatment. This partially explains the observation of no significant correlation between the level of CK1α and ARSI treatment probability (data not shown). However, fully inhibiting CK1α with A51 tips this balance toward cell death. Upon combination treatment of A51 with ENZA, cells will undergo apoptosis due to ATM stabilization-initiated extensive DSB-response signaling, including the p53 pathway, regardless of the level of β-catenin or Wnt activity.

Previous studies showed that AR and AR variants were involved in DNA repair progression in PCa cells[33, 34]. ENZA treatment could suppress the expression of HR genes, and induce HR deficiency and "BRCAness"[25], a phenotypic trait that cells share with characters of BRCA1/2 mutation carriers, which subsequently promote DNA damage-induced cell death. In congruence with these findings, we observed that patients with a high expression level of AR displayed a high activity of gene sets in DNA DSB repair (Extended Data, Figs. 6f-6h), and that ENZA treatment induced DSB response in ENZA-sensitive cells. Patients or cells categorized as non-responsive to ENZA showed a lower activity of DSB signaling, but restoration of DSB-response signaling by inhibition of CK1α rendered otherwise resistant cells sensitive to ENZA. In addition, CK1α inhibition could decrease AR activity (data not shown), which will further stimulate the DSB response and cell death. Our findings further solidify DDR signaling as an essential player in the ENZA response and identify a therapeutic target to halt disease progression.

Kinases ATM, ATR and DNA-PKcs play pivotal roles in DDR by phosphorylating a variety of effectors to control DNA repair, cell cycle arrest or cell death in response to DNA damage[35]. In contrast to ATR and DNA-PKcs, ATM serves as a central node in response to DNA DSB[36]. ATM must be tightly regulated as aberrant activation of ATM could lead to toxic DNA repair, cell-cycle arrest, senescence, or apoptosis[35]. We showed that ATM was a direct substrate of CK1α in regulation of DNA DSB signaling and the ENZA response. It has been well established that p53 abundance is regulated by CK1α, whereas ATM is an important regulator of p53 activity and stability in a direct or indirect manner[35, 37]. Therefore, our findings expand our knowledge of the scale of CK1α in the regulation of DDR and offer another mechanism of the regulation of p53 by CK1α through the modulation of the abundance of ATM.

As described above, DSB signaling is important in ENZA-induced cell death. As the primary initiator of the DSB response, ATM is significant in this process. Patients with a high expression level of ATM showed a significantly increased time frame to progression on ARSIs treatment compared with patients with a lower level of ATM. A more recent clinical study reported that ATM and BRCA2 were surprisingly

differentially gained in ENZA-resistant versus sensitive CTCs DNA, with BRCA2 gain enriched in resistant patients and ATM gain enriched in sensitive patients[24]. ENZA could induce “BRCAness”[25], which would subsequently lead to ATM-dependent cell death, whereas BRCA2 gain would attenuate ENZA-induced “BRCAness” and cell death. Therefore, ATM and BRCA2 seem to play oppositive roles in the ENZA response. This also explains why alterations of DNA-damage genes (including ATM and BRCA1/BRCA2) are not correlated with ENZA treatment probability[23], whereas the expression level of ATM itself is.

Lineage plasticity has been implicated in drug resistance, particularly molecularly targeted therapies such as ARSI. In PCa, loss of p53, RB1, and androgen-dependent signaling are the hallmarks of lineage plasticity or neuroendocrine PCa, which has shown strong resistance to ARSI[38–40]. At present, there is no established therapeutic approach for these patients. Intriguingly, in addition to DDR signaling, androgen response gene set was also significantly negatively enriched in patients with high expression of CK1α compared with patients with a lower level of CK1α (Extended Data Fig. 6i), indicating that CK1α inhibition might be able to convert cell lineage from androgen-independent to androgen-dependent. Furthermore, as a well-established negative regulator of p53[31], inhibition of CK1α will increase p53 abundance. These observations suggest that CK1α might also be a target for lineage plasticity PCa therapy through modulation of p53 and androgen-dependent signaling. Further experiments need to be conducted to validate and establish an accurate model.

Materials And Methods

Kinome-wide CRISPR-Cas9 screen.

22Rv1 cells were transduced with lentiCas9-Blast (addgene, #52962) and then selected with 10 μg ml^{-1} blasticidin for 3 weeks to generate a stable cell line expressing Cas9 (22Rv1-Cas9). Single clones were picked and further determined Cas9 expression and ability to edit genes knockout.

Human Kinome CRISPR pooled library (Brunello)[41] was a gift from Drs. John Doench and David Root (addgene #75314). The library was amplified as previously described[41]. Briefly, the Kinome library was diluted to 50 ng ul^{-1} in water and then electroporated using Endura electrocompetent cells (Lucigen, #60242). The transformations were plated onto pre-warmed agar plates and incubated for 14 hrs at 32 °C. Colonies were harvested and the library plasmids were purified with a maxi scale plasmid prep (Qiagene, #12162).To make lentivirus, the library plasmids were co-transfected with packaging plasmids pCMV-VSV-G (addgene, #8454) and psPAX2 (addgene, #12260) into HEK293T cells with Lipofectamine 2000 (Thermo Fisher). After 6 hrs, the medium was changed and further cultured for an additional 60 hrs, then viral supernatants were harvested and centrifuged at 2,000 rpm at 4 °C for 10 min to pellet cell debris. The supernatant was then filtered through a 0.45 μm membrane and viruses were concentrated with a high speed centrifuge (24, 000 rpm, 2 hrs). The titer of the lentiviruses was determined with 22Rv1 cells in a functional assay by measuring puromycin resistance after transduction.

Infections were set up at 1500-fold coverage of the library. 22Rv1-Cas9 cells were infected at a low multiplicity of infection (MOI = 0.3) with the above prepared kinome library viruses in the presence of 8 μg ml^{-1} polybrene, followed by selection with puromycin for 3 days, then 1/3 of the infected cells (500 × coverage) were harvested as the baseline for deep sequencing and the remaining cells were continuously cultured for an additional two weeks waiting for genes edition. Then the cells were randomly split and treated with 20 μM ENZA or DMSO for an additional one week. The sgRNAs incorporated into the cells were amplified from genomic DNA and sequenced on Illumina HiSeq 2500.

Analysis of CRISPR-Cas9 screen data

Screen data analysis was performed by MAGeCK[42] software. Raw sequencing reads were processed by FastQC for quality control, 21-27 bp were trimmed from the 5' end until the protospacer sequence was reached, then mapped to the expected sgRNA sequence with zero mismatches tolerated. The read counts of sgRNAs were normalized by non-targeting control sgRNAs. Using the MAGeCK MLE algorithm, the normalized sgRNA read counts in ENZA and DMSO samples were compared to the initial baseline sgRNA distribution and negative control sgRNAs to estimate a beta score for each targeted gene in the treatment and control groups separately. The beta scores of all genes were normalized by the median beta score of the essential genes to make the cell proliferation rates comparable between treatment and control samples and to reduce false-positive hits [17]. The top negatively selected genes were those with the smallest negative values in the differential beta score, which was calculated by subtracting the control beta score from the treatment beta score.

RNA sequencing and analysis

Total RNA derived from 22Rv1, 22Rv1 with CK1α knockout and 22Rv1 cells with CK1α overexpression was purified using an RNeasy Mini kit (Qiagene, #74104). The RNA sequencing was run as a paired-end 150bp in length with 30 million reads, on the Illumina HiSeq 2500 (Novogene). Raw sequences were mapped via HISAT2[43] (v2.1.0) to the human reference genome GRCh38. Read counting was performed using featureCounts[44] from the subread package (v1.5.1). Differential gene expression was analyzed by R package limma[45] (v3.42.2). All p-values were corrected for multiplicity by the Benjamini-Hochberg method. Gene Set Enrichment Analysis (GSEA) statistical analysis was carried out with publicly available software from the Broad Institute (http://www.broadinstitute.org/gsea/index.jsp). Gene expression results were ranked and used to conduct GSEA. GSEA was performed to evaluate enrichment of differential expression patterns curated from Hallmarks, Reactome, BioCarta, and KEGG database within the MSigDBv7.4.

RNA sequencing of C4-2 and C4-2R cells has been described[46]. Briefly, extracted total RNA from C4-2 and C4-2R cells was sequenced as a paired-end 100bp in length with 30 million reads, on the Illumina HiSeq 2500 (Purdue Genomics Facility). Tophat2 was used to align reads to the Ensembl Homo sapiens genome database version GRCh38.p5. The htseq-count script in HTSeq v.0.6.1 was run to count the number of reads mapping to each gene. DESeq2, edgeR and Cufflinks2 were used for differential

expression analysis. Gene expression results were ranked and used to conduct GSEA. GSEA was performed accordingly.

Cell culture

Human PCa cell lines LNCaP, C4-2, MR49F, C4-2R and 22Rv1 were cultured in RPMI-1640 medium (Sigma-Aldrich). Among these cell lines, LNCaP is androgen-dependent, whereas C4-2 is an androgen-independent cell line derived from LNCaP. MR49F is an ENZA-resistant derivative of LNCaP obtained from Dr. Amina Zoubeidi (University of British Columbia, Vancouver, BC, Canada), whereas C4-2R is derived from C4-2 and kindly provided by Dr. Allen Gao (University of California, Davis, CA, USA). The concentration of ENZA for ENZA-resistance maintenance of MR49F and C4-2R was 10 or 20 μM, respectively. ENZA was removed for at least 48 hrs before any experiment. 22Rv1 is an established intrinsic ENZA resistant cell line. LNCaP, C4-2, and 22Rv1 cells were purchased from American Type Culture Collection. HEK293T cell was a gift from Dr. Andrea Kasinski (Purdue University, USA) and cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich). All medium contained 10% fetal bovine serum (Atlanta Biologicals, GA, USA), 100 U ml^{-1} of penicillin and 100 μg ml^{-1} streptomycin. Cells were cultured in a humidified atmosphere at 37 °C, with 5% CO_2.

Mouse models and treatment

All animal experiments were approved by the Institutional Animal Care and Use Committee at University of Kentucky (KY, USA). Patient derived xenograft (PDX) experiments were conducted as previous described[21]. Male NSG (NOD scid gamma) mice (age 6-8 weeks) were castrated and then implanted subcutaneously with LuCaP 35CR tumor bits two weeks later. When the tumor volume reached 50 mm^3, mice were pre-treated with ENZA (50 mg kg^{-1} in PBS containing 1% carboxymethyl cellulose, 0.1% Tween 80, 5% DMSO) by oral gavage daily, for a routine of 5 days on, 2 days off. After one week, ENZA resistance would have developed[21]. Mice were randomly assigned into four groups: Control (with vehicle); ENZA (20 mg kg^{-1} in PBS containing 1% carboxymethyl cellulose, 0.1% Tween 80, 5% DMSO, by oral gavage every 3 days); A51 (5 mg kg^{-1} in ddH_2O containing 10% DMSO, 10% Solutol and 10% 2-hydroxy prolyl-b-cyclodextrin, by oral gavage, two days on and one day off); or combination with ENZA and A51 (Figure 2G). Tumor growth was monitored twice weekly by measuring the length and width of tumors. Tumor volume was evaluated using the formula: (length × $width^2$)/2. The mice were sacrificed when tumor volume exceeded 1,500 mm^3, when the diameter exceeded 20 mm, when the animals became compromised, or after 12 weeks, whichever developed first for the end point of the study. After humane euthanization, the tumors were harvested for paraffin embedding or frozen for subsequent analyses.

22Rv1 *in vivo* xenograft experiments were conducted by subcutaneous injection of 2 × 10^6 22Rv1 cells (wild-type or CK1α-knockout) (100 μl in 50% PBS and 50% Matrigel, Corning) into the flanks of pre-castrated male nude mice (Jackson Lab, MI, USA). When the tumor volume reached 100 mm^3, daily gavage treatment with 20 mg kg^{-1} ENZA or vehicle (PBS containing 1% carboxymethyl cellulose, 0.1%

Tween 80, 5% DMSO) was started and continued for 5 weeks (5 days on, 2 days off) (Figure 2D). Tumor growth was monitored twice weekly by measuring the length and width of tumors. Tumor volume was evaluated using the formula: (length × width^2)/2. After humane euthanization, tumors were harvested for paraffin embedding or frozen for subsequent analyses.

Reagents

Enzalutamide (S1250), doxycycline (S5159), cycloheximide (S7418), MG132 (S2619) and D4476 (S7642) were purchased from Selleck Chemicals (TX, USA). A51 was synthesized by DC Chemicals (Shanghai, China). CK1α kinase (PV3850) was purchased from Thermo Fisher (MA, USA). ATM protein (14-933) and polybrene (TR-1003-G) were purchased from Sigma-Aldrich (MO, USA). Transfection Reagents Lipofectamine 2000 (11668019) and jetPRIME® (89129-924) were obtained from ThermoFisher (MA, USA) and VWR (PA, USA), respectively. For *in vitro* study, chemicals were dissolved in DMSO (D2650, Sigma-Aldrich). Enzalutamide used in animal studies was dissolved in PBS containing 5% DMSO (D2650, Sigma-Aldrich), 1% carboxymethyl cellulose (S6703, Selleck Chemicals) and 0.1% Tween 80 (P4780, Sigma-Aldrich) as previously described[21]. A51 was dissolved in ddH_2O containing 10% DMSO (D2650, Sigma-Aldrich), 10% Solutol (HY-Y1893, MedChemExpress) and 10% 2-hydroxy prolyl-b-cyclodextrin (HY-101103, MedChemExpress) as previously described [26]. Protein A/G Magnetic Beads (#88802) were purchased from ThermoFisher (MA, USA).

Plasmids

The following plasmids were obtained from Addgene: lentiCas9-Blast (#52962), lentiGuide-Puro (#52963), LentiGuidPuro-hTP53 (#88853), sgTP53_3 (#78164), pcDNA-FLAG-ATM (#43907), PRKDC gRNA1 (#77861), PRKDC gRNA2 (#77862), ATM gRNA1 (#77530), ATM gRNA2 (#77531), CHEK2 gRNA1 (#76487), CHEK2 gRNA2 (#76488), Tet-plko-puro (#21915), pLC-Flag-CSNK1A1-WT-Puro (#123319), CSNK1A1 gRNA1 (#76188), CSNK1A1 gRNA2 (#76189), pcDNA3.1-HA (#128034), psPAX2 (#12260) and pCMV-VSV-G (#8454). Myc-DDK-CK1α (#RC217936) was purchased from OriGene (MD, USA). GST-ATM fragments were gifts from Dr. Zhenkun Lou (Mayo Clinic, Rochester, USA) and site mutations were generated by a Q5 Site-Directed Mutagenesis Kit (New England Biolabs, E0554). PcDNA3.1-HA-CK1α, Tet-plko-puro-CK1α, sgAR-full length were generated in this study.

Antibodies

Antibodies for immunobloting: anti-Cas9 mAb (#14697), anti-AR mAb (#5153), anti-β-Actin mAb (#4970), anti-CK1α mAb (#2655), anti-ATM mAb (#2873), anti-CHK2 Ab (#2662), anti-phospho-CHK2 (T68) Ab (#2661), anti-γH2AX mAb (#9718), anti-H2AX mAb (#7631), anti-DNA-PKcs mAb (#38168), anti-Cleaved PARP (Asp214), anti-Myc-Tag (#2278), anti-HA-tag (#3724) were purchased from Cell Signaling Technology (MA, USA). Anti-vinculin mAb (v4505) and anti-Flag-tag (F1804) were bought from Sigma-Aldrich (MA, USA). Anti-CK1α mAb (sc74582) and anti-p53 mAb (sc126) were from Santa Cruz Biotechnology (CA, USA). Antibodies for immunoprecipitation: anti-HA Magnetic Beads (#88836) was obtained from ThermoFisher (MA, USA), anti-Flag Magnetic Beads (M8823) was purchased from Sigma-

Aldrich (MA, USA). Anti-CK1α mAb (sc74582) was from Santa Cruz and anti-ATM mAb (ab201022) was purchased from Abcam (MA, USA). Antibodies for immunofluorescence (IF) and immunohistochemistry (IHC) assay: anti-γH2AX (#9718), anti-Cleaved Caspase-3 (#9661) and anti-phospho-CHK2 mAb (T68) (#2197) were obtained from Cell Signaling Technology. Anti-CK1α (TA313698) was obtained from Origene (MD, USA). Anti-ATM (ab32420) was from Abcam (Cambridge, UK). Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488(A-11034) was purchased from Thermo Fisher (MA, USA). Anti-phospho-ATM (S1270) pAb was made by Sino Biological (PA, USA).

Tumor microarray (TMA) construction and IHC staining

For TMA, formalin-fixed paraffin-embedded tissue blocks from prostate cancer patients who underwent radical prostatectomy at the University of Kentucky (KY, USA) were cored to construct a TMA through the Markey Cancer Center Biospecimen Procurement and Translational Pathology Shared Resource Facility. Approval for use of human prostate tissue was obtained from the University of Kentucky Institutional Review Board. Cancerous, adjacent benign epithelial, or benign prostatic hyperplasia tissue cores (2 mm) were used, with duplicate cores from each patient. Four-micron thick sections of the TMA were cut for IHC staining. For regular xenograft tumor, four-micron thick sections of formalin-fixed paraffin-embedded tumor tissue were cut for IHC staining. Staining was carried out on Ventana Discover Ultra. Antigen retrieval was performed using CC1 (Roche) (for ATM, γH2AX and Cleaved Caspase-3) or CC2 (Roche) (for CK1α) with standard conditions. Slides were incubated with primary antibodies (ATM at 1:50, γH2AX at 1:100, Cleaved Caspase-3 at 1:150 and CK1α at 1:50) for 1 hr at 37 °C, incubated with anti-Rabbit-HQ (Roche), followed by anti-HQ-HRP (Roche) and visualized with DAB prior to light counterstain with Mayer's hematoxylin. The TMA staining was evaluated and scored by a pathologist. The regular staining of tumor xenografts was also evaluated by a pathologist by comparison to the positive control. Images were taken with a Nikon microscopic camera or scanned with the Aperio Digital Pathology Slide Scanner.

Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde for 15 min at room temperature (RT), permeabilized in 0.2% Triton X-100 for 10 min and blocked with 10% FBS/PBS (v/v) for 30 min. Afterwards, the cells were incubated with appropriate primary antibodies overnight at 4°C and secondary antibodies for 1 hr at RT. All antibodies were diluted in PBS containing 1% BSA and 1% normal goat serum. The dilution ratios of the primary antibodies were 1:500 for ATM and γH2AX, 1:100 for p-CHK2. The secondary antibody was used at 1: 500. The nucleus was stained with Hoechst (Sigma-Aldrich, B2261). Images were captured with a confocal microscope (Nikon).

Immunoblot (IB) and immunoprecipitation (IP) analyses

Cell lysates were prepared as previously described[47]. Briefly, cultured cells were harvested and lysed with the lysis buffer (50 mM Tris, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.2% SDS, protease inhibitors, and phosphatase inhibitors). For tumor tissue, the lysates were prepared by homogenization using the above lysis buffer followed by sonication with a probe sonicator. After quantification with BCA

assay, equal amounts of protein were loaded on SDS-PAGE gel and then transferred to nitrocellulose membranes, followed by IB with the indicated antibodies. For IP analysis, 1 mg protein collected with the lysis buffer (50 mM Tris, 150 mM NaCl, 0.5% sodium deoxycholate,1% NP-40, protease inhibitors) were incubated with the indicated primary antibodies overnight at 4°C followed by the protein A/G magnetic beads for 3 hrs at RT or antibody-conjugated beads overnight at 4 °C. The immunocomplexes were washed three times with the lysis buffer and analyzed by IB.

AquaBluer assay to determine cell viability

AquaBluer assay was performed according to the manufacturer's instructions (MultiTarget, # 6015). Cells were seeded at 3000-8000 (dependent on the cell size) per well in 96-well culture plates for cells grow overnight. Cells were then treated as indicated and further cultured for 72 hrs. Dilute AquaBluer™ (1: 100) in the culture medium and mix well. The medium was then removed from the cell culture and 100 µl of the diluted AquaBluer™ was added to each well. The plate was returned to the incubator for another 4 hrs. Once placed in a fluorescence plate reader, the fluorescence intensity was read at 540ex/590em.

Colony formation assay

Cells were seeded at 500-2000 (dependent on cell size) per well in 6-well or 12-well culture plates. After growing for 24 hrs, cells were treated as indicated and further cultured for 3 weeks. The medium was then removed and the cells were rinsed carefully with PBS. Cells were fixed with 4% paraformaldehyde for 15 min at RT before they were stained with 0.5% crystal violet for 30 min at RT. After staining, the crystal violet solution was removed and rinsed carefully with tap water. Colonies were left to dry on the plates at RT. The images were taken with the ChemiDoc Imaging System (Bio-Rad).

In vitro kinase assay

In vitro kinase assays were performed with a kinase buffer (CST, #9804) (25 mM Tris-HCl (pH 7.5), 5 mM beta-glycerophosphate, 2 mM dithiothreitol (DTT), 0.1 mM Na_3VO_4 and 10 mM $MgCl_2$) supplemented with 125 µM ATP and 10 µCi of [γ- 32P] ATP at 30 °C for 30 min in the presence of CK1a kinase and GST-ATM proteins. After the reaction, mixtures were resolved by SDS-PAGE, the gels were stained with Coomassie brilliant blue, dried, and subjected to autoradiography. *In vitro* kinase assays for mass spectrometry analysis were performed with the kinase buffer above supplemented with 200 µM ATP (CST, #9804) at 30 °C for 30 minutes in the presence of CK1a kinase and GST-ATM proteins. After the reaction, proteins were denatured, resolved on SDS–PAGE, the gels were stained with Coomassie brilliant blue, the protein bands were cut and subjected to mass spectrometry analysis.

sgRNA or shRNA clones

To clone the sgRNA or shRNA to the vectors, lentiGuide-Puro (Addgene #52963) or Tet-plko-puro (Addgene #21915) was cut by restriction enzymes and dephosporylated with FastAP (Thermo Fisher). Oligonucleotides for the sgRNAs or shRNAs sequence (sgAR-full#1 5'GTTACACGTGGACGACCAGA3'

sgAR-full#2 5'GTGTCCAGCACACACTACACC3', shCK1α 5'GCCACAGTTGTGATGGTTGTT3') were phosphorylated using polynucleotide kinase and then annealed by heating to 95 °C for 5 minutes and cooling to 25 °C at 1.5 °C minute^{-1}. Annealed oligos were then ligated into lentiGuide-Puro or Tet-plko-puro above using T4 ligase (New England Biolabs) at 16 °C overnight.

Statistics Methods

For comparisons between two groups of independent datasets, multiple t-tests were performed, and p-value and standard error of the mean (SEM) were reported. For comparisons among more than two groups, one-way or two-way ANOVA were performed, p-values and SEM were reported. For drug response curves, p-values were calculated by non-linear regression with paired t-tests. For all figures, $*$ represents $p<0.05$, $**$ represents $p<0.01$, $***$ represents $p<0.001$. All bioinformatic analysis and comparisons are described below.

Bioinformatic analysis

Correlation analysis with Spearman's Correlation coefficient

The Spearman's Correlation coefficient and its associated 95% confidence interval (CI) were used to quantify the correlation between ATM and CK1α expression in TMA IHC staining. A linear regression line helps visualize the negative correlation between the two genes.

Analysis of "SU2C PNAS2019" dataset

Scatter plot together with Spearman's rank correlation coefficient was applied to evaluate the correlation strength between the duration of ARSI treatment and FPKM-normalized ATM mRNA expression based on the "SU2C PNAS2019" datasets[23]. A linear regression line helped visualize the correlation. The analysis concerns only those patients whose ARSI treatment status is recorded as "Off treatment"=TRUE. SU2C PNAS-2019 mRNA FPKM expression values were downloaded from the cBioportal website (https://www.cbioportal.org/). The histogram of the FPKM-normalized gene levels of ATM of the SU2C PNAS-2019 samples was plotted to demonstrate the distribution of ATM levels. A dashed line indicates the median expression of ATM. Kaplan-Meier curve, together with the logrank p-value, was generated to evaluate the association between the possibility of patients staying on the line of ARSI treatment and the ATM expression levels (dichotomized as high versus low with median chosen as the cutoff). Forest plot associated with the statistics based on Cox proportional hazards model was generated to evaluate the association between the ARSI-treatment duration length and the ATM mRNA levels (dichotomized as high versus low with median chosen as the cutoff). An Hazard Ratio being less than 1 indicates that patients in the ATM-high group tended to remain on the line of ARSI treatment longer than those in the ATM-low group.

GSEA analysis with TCGA dataset (https://www.cancer.gov/tcga)

TCGA-PRAD mRNA FPKM expression values were downloaded from the GDC data portal and manually converted to TPM-normalized gene levels. The histogram of the interested gene level from the TCGA-PRAD tumor samples was plotted to demonstrate the distribution of gene expression. The dashed lines indicate the median or quartile expression of the interested genes. Differential comparison analysis between the indicated gene expression high (>= cut off, indicated in the figure legend) and the low (< cut off) subgroups was performed based on the quasi-likelihood F-test in R package edgeR. Genes were then ranked based on p-values together with the corresponding signs of log2-foldchange. Pre-ranked GSEA was then performed accordingly.

Analysis of "Dr. Alumkal PNAS2020" dataset

Dr. Joshi Alumkal kindly provided the raw count table from their RNA-seq dataset[7]. Raw read counts were log transformed and analyzed by limma[45] to generate log2 fold change for non-responders versus responders, which was used to pre-rank the genes as the input for GSEA. A CK1α gene signature composed of 188 genes was generated by overlapping up regulated genes (adjusted $p<0.05$, knockout/control ratio >1) in CK1α knockout samples and down regulated genes (adjusted $p<0.05$, overexpression/control ratio <1) in CK1α overexpression samples. Heatmap was generated by R package pheatmap.

Declarations

Author Contributions

J.L. designed, performed experiments, analyzed the data and wrote the manuscript. X.L. designed the research and supervised the project. Y. Zhao, D.H., S.T., C.L., C.W. and L.L. performed the analysis of CRISPR-screening, RNA-seq and other bioinformatics. K.J. conducted manuscript editing. D.A. evaluated and scored the IHC staining. Y. Zhang conducted the amplification of the CRISPR library. J.C. performed mass spectrometry assay and analyzed the data. K.J., Q.Z. and X.W. conducted xenograft tumors harvesting and some data analysis.

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Figures

Figure 1

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Figure 2

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Figure 4

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Figure 5

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Figure 6

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Figure 7

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ExtendedDataTable1.xlsx
- ExtendedDataTable2.pdf
- ExtendedDataTable3.xlsx