remembered for ~2 weeks, although extinction is rapid once animals learn that the involatile pheromone is no longer present. This suggests that darcin is a particularly salient social cue for attracting mice of both sexes. It appears to activate a specific mechanism of associative learning so that instinctive attraction to spend time near this pheromone is extended both to its learned location and to airborne odors associated with the pheromone (*15*). Single-trial learning of associated odors is induced by another pheromone from rabbit mammary glands to improve pup ability to localize nipples efficiently (*22*), but spatial learning is unlikely to be involved.

This establishes a new role for mammalian pheromones in stimulating learned as well as instinctive social responses. Pheromone-induced learning may be much more important than previously recognized, allowing animals to remember and rapidly relocate scent-marked sites of particular social relevance and driving the flexible individual-specific social responses that typify mammals. Even though all adult male mice produce the same sex pheromone, pheromone-induced learning strongly reinforces attraction to a particular individual male and his location. Learned attraction to the individual-specific airborne odor associated with darcin further targets attraction to other scent marks emitting the same individual's odor, resulting in contact with darcin and conditioned preference for other scent-marked sites as well as to the individual male himself. Thus, pheromone-induced learning reinforces attraction to a particular male much more effectively than does simple attraction to the pheromone alone. The reliable and rapid learning induced by darcin among both female and male mice provides a valuable and tractable new model to investigate the neural pathways and mechanisms involved in spatial learning and in the learning of complex individual-specific social odors in response to a specific pheromone stimulus. It may also help to establish how such social information about individual conspecifics is stored and integrated in the brain.

References and Notes

1. R. E. Brown, D. W. Macdonald, *Social Odours in Mammals* (Clarendon Press, Oxford, 1985).
2. J. L. Hurst, R. J. Beynon, *Bioessays* **26**, 1288 (2004).
3. L. M. Gosling, *J. Comp. Ethol.* **60**, 89 (1982).
4. J. L. Hurst, *Behav. Brain Res.* **200**, 295 (2009).
5. L. M. Gosling, S. C. Roberts, *Adv. Stud. Behav.* **30**, 169 (2001).
6. T. Wyatt, *Pheromones and Animal Behaviour* (Cambridge Univ. Press, Cambridge, 2003).
7. E. D. Morgan, *Physiol. Entomol.* **34**, 1 (2009).
8. K. Johannesson *et al.*, *Evolution* **62**, 3178 (2008).
9. M. P. LeMaster, I. T. Moore, R. T. Mason, *Anim. Behav.* **61**, 827 (2001).
10. R. G. Paredes, *ILAR J.* **50**, 15 (2009).
11. M. R. Bell, S. H. Meerts, C. L. Sisk, *Horm. Behav.* **58**, 410 (2010).
12. D. E. Pankevich, J. A. Cherry, M. J. Baum, *Physiol. Behav.* **87**, 781 (2006).
13. J. Martínez-Ricós, C. Agustín-Pavón, E. Lanuza, F. Martínez-García, *Chem. Senses* **32**, 139 (2007).
14. S. A. Ramm, S. A. Cheetham, J. L. Hurst, *Proc. Biol. Sci.* **275**, 1727 (2008).
15. S. A. Roberts *et al.*, *BMC Biol.* **8**, 75 (2010).
16. S. A. Cheetham, A. L. Smith, S. D. Armstrong, R. J. Beynon, J. L. Hurst, *Physiol. Behav.* **96**, 253 (2009).
17. F. J. Schwende, D. Wiesler, J. W. Jorgenson, M. Carmack, M. Novotny, *J. Chem. Ecol.* **12**, 277 (1986).
18. S. Harvey, B. Jemiolo, M. Novotny, *J. Chem. Ecol.* **15**, 2061 (1989).
19. D. Y. Lin, S. Z. Zhang, E. Block, L. C. Katz, *Nature* **434**, 470 (2005).
20. J. Moncho-Bogani, E. Lanuza, A. Hernández, A. Novejarque, F. Martínez-García, *Physiol. Behav.* **77**, 167 (2002).
21. J. M. Mudge *et al.*, *Genome Biol.* **9**, R91 (2008).
22. G. Coureaud *et al.*, *Curr. Biol.* **16**, 1956 (2006).

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Supplementary Materials

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EZH2 Oncogenic Activity in Castration-Resistant Prostate Cancer Cells Is Polycomb-Independent

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Epigenetic regulators represent a promising new class of therapeutic targets for cancer. Enhancer of zeste homolog 2 (EZH2), a subunit of Polycomb repressive complex 2 (PRC2), silences gene expression via its histone methyltransferase activity. We found that the oncogenic function of EZH2 in cells of castration-resistant prostate cancer is independent of its role as a transcriptional repressor. Instead, it involves the ability of EZH2 to act as a coactivator for critical transcription factors including the androgen receptor. This functional switch is dependent on phosphorylation of EZH2 and requires an intact methyltransferase domain. Hence, targeting the non-PRC2 function of EZH2 may have therapeutic efficacy for treating metastatic, hormone-refractory prostate cancer.

Factors involved in maintaining the epigenetic state of the cell are frequently altered in cancer and are promising therapeutic targets. The expression of EZH2 (enhancer of zeste homolog 2) is correlated with prostate cancer progression, especially to its lethal castration-resistant state (CRPC) (*1*). EZH2 is the catalytic subunit of Polycomb repressive complex 2 (PRC2), which silences transcription through trimethylation of Lys^{27} on histone H3 (H3K27me3) (*2*). Most studies have focused on PRC2-mediated repression as the oncogenic mechanism of EZH2. In addition, tumor suppressors such as *DAB2IP* have been reported as EZH2 or PRC2 targets (*3*). However, substantial studies have indicated that both *Drosophila* E(z) (enhancer of zeste) and EZH2 have potential functions other than that of a transcriptional repressor (*4*–*6*), although the mechanisms are unclear.

We used the LNCaP cell line as a model of androgen-dependent prostate cancer and LNCaP-abl (abl), its androgen-independent derivative (*7*), to study EZH2 function in the progression of prostate cancer to CRPC. As is the case for clinical tumors (*1*), EZH2 levels in abl cells were much higher than in LNCaP cells (Fig. 1A). EZH2 silencing had a more profound effect on the androgen-independent growth of abl cells than on the androgen-dependent growth of LNCaP cells (Fig. 1B and fig. S1). The requirement of EZH2 for androgen-independent growth was confirmed in an in vivo mouse xenograft CRPC model using CWR22Rv1 cells (Fig. 1C).

Next, we explored EZH2-dependent genes in LNCaP and abl cells. Although similar numbers of genes were up- or down-regulated after EZH2 silencing in LNCaP cells, many more genes were significantly down-regulated upon EZH2 depletion in abl cells, and these EZH2-stimulated genes were highly expressed in abl cells (Fig. 1D). EZH2 silencing by means of two independent small interfering RNAs (siRNAs) confirmed the derepression of the EZH2-repressed gene *DAB2IP* in LNCaP cells and the down-regulation of several EZH2-stimulated genes in abl cells (fig. S2A). We found similar results in two other hormone-refractory cell lines, C4-2B and CWR22Rv1 (fig. S2B). We then examined

the profiles of EZH2-dependent genes in two clinical prostate cancer cohorts (*8*, *9*). Although the set of EZH2-repressed genes in LNCaP cells exhibited lower expression in CRPC and marginal negative correlation with EZH2 level, the set of EZH2-stimulated genes identified in abl cells had significantly higher expression levels and positive correlation with EZH2 in these metastatic, hormone-refractory prostate tumors (Fig. 1, E and F, and fig. S3). These results suggest a potentially important functional switch of EZH2 from transcriptional repression to gene activation in CRPC.

To determine whether the gene activation function of EZH2 is the effect of direct binding, we conducted chromatin immunoprecipitation sequencing (ChIP-seq) of EZH2 and H3K27me3. Although EZH2 and H3K27me3 colocalized at the majority of sites in both LNCaP and abl cells, we identified a subset of EZH2 sites that lack nearby H3K27me3 in abl cells (Fig. 2A). These EZH2 sites lacking H3K27me3 were validated with the use of four different EZH2 antibodies (fig. S4A) and by EZH2 silencing (fig. S4B). We defined EZH2 "ensemble" peaks as those with both EZH2 and H3K27me3 enrichment, and "solo" peaks as those with only EZH2 binding. A majority of both ensemble and solo binding sites were located at the promoter regions or gene bodies (fig. S5A). Although ensemble peaks in LNCaP and abl cells overlapped considerably, very few solo peaks overlapped between the two cell lines (fig. S5B). The genes near EZH2 binding sites displayed even more striking differences (fig. S5C). This finding suggests that EZH2 gains a unique set of chromatin binding sites that lack H3K27me3 in abl cells. In addition, the solo peaks were enriched for the active histone marks H3K4me2 and H3K4me3 (i.e., dimethylation and trimethylation of Lys^4 on histone H3) and RNA polymerase II (Pol II) (Fig. 2B), suggesting the potential function of these peaks in gene activation. Indeed, although ensemble binding was enriched near the transcription start sites of EZH2-repressed genes, solo binding was enriched near EZH2-stimulated genes (Fig. 2C and fig. S6). Strikingly, EZH2 depletion decreased the levels of the active marks at these solo sites (fig. S7), indicating a mechanism of EZH2 in gene activation via modulation of active chromatin states. Genes directly activated by EZH2 in abl cells were significantly overexpressed in gene signatures derived from independent metastatic, hormone-refractory prostate tumors (fig. S8A), and survival analysis supported the prognostic power only of EZH2-activated genes with solo peak binding in abl cells [Fig. 2D (*8*) and fig. S8, B to D (*10*)]. Taken together, these data support the importance of EZH2 gene activation function in CRPC.

We then tested the involvement of another PRC2 subunit, SUZ12, in the EZH2 solo peaks in CRPC. SUZ12 ChIP-seq signals displayed strong correlation with both H3K27me3 (0.57) and EZH2 ensemble peaks (0.66), but little correlation with EZH2 solo peaks (0.27) (fig. S9A). Silencing of SUZ12 drastically reduced EZH2 binding at ensemble peaks but had no effect at the solo peaks (fig. S9B). Silencing of either SUZ12 or another PRC2 core component, EED, increased the expression of the EZH2-repressed gene *DAB2IP* but had no effect on EZH2-

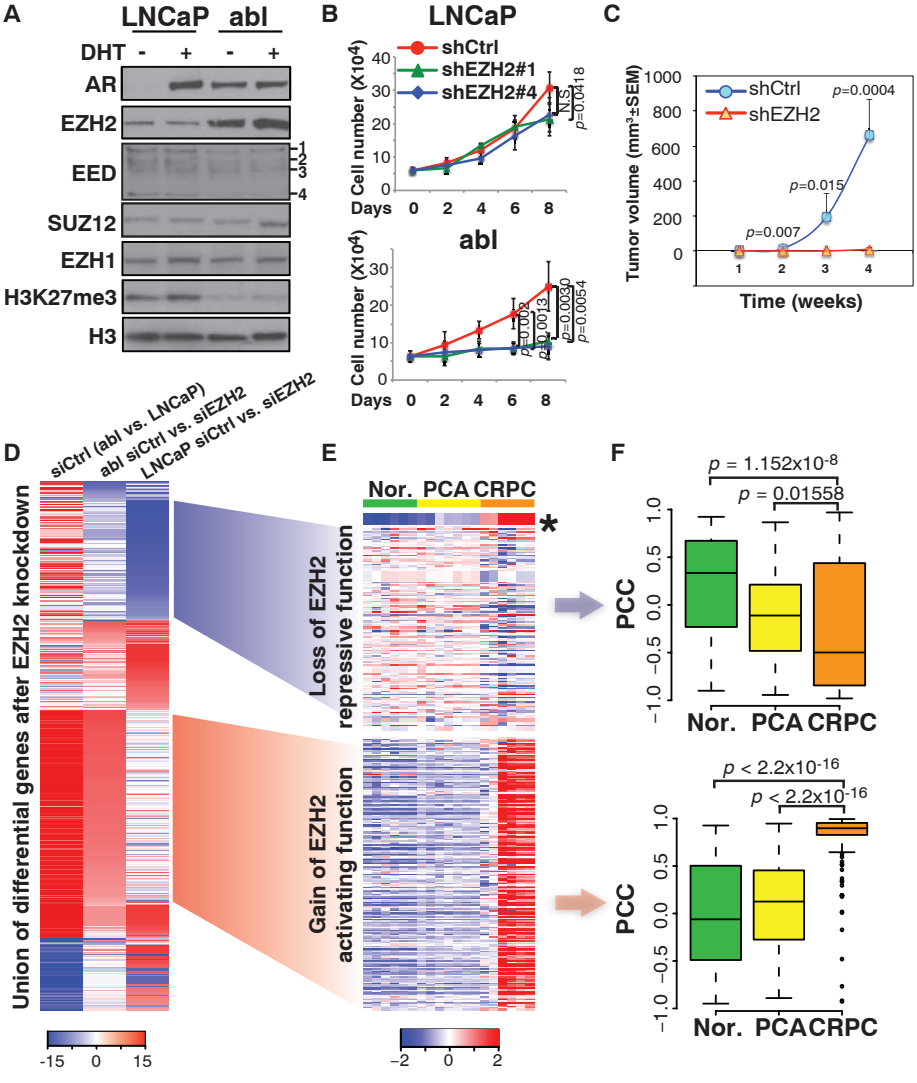

Fig. 1. Overexpression of EZH2-stimulated genes in clinical CRPC samples. (**A**) Immunoblot of nuclear extracts from LNCaP and abl cells treated without (–) or with (+) 5α-dihydrotestosterone (DHT) to control for effects of cell growth on protein expression. EED isoforms are numbered (*22*). EZH1, enhancer of zeste homolog 1. (**B**) Growth of cells transduced with lentiviral short hairpin RNAs (shRNAs) targeting scrambled control (shCtrl) or EZH2 (shEZH2#1 and #4). (**C**) Tumor growth curve of castrated male *scid* mice injected with CWR22Rv1 cells with or without EZH2 silencing. (**D**) Clustering of the union of differentially expressed genes in LNCaP and abl cells after transfection with siRNAs against control (siCtrl) or EZH2 (siEZH2). (**E** and **F**) Heat map of expression levels (E) and box plots of Pearson correlation coefficients (PCC) (F) of EZH2-repressed (top) and EZH2-stimulated (bottom) genes with EZH2 level in the Varambally *et al.* (*9*) cohort. Nor., normal tissues; PCA, primary tumors. Asterisk in (E) denotes EZH2 level.

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activated genes (fig. S9C). These results indicate that EZH2 solo peaks are independent of the PRC2 complex. Results from gel filtration chromatography demonstrated that EZH2 is present in complexes other than PRC2 in abl cells, because a large fraction of EZH2 eluted as a broad peak distinct from SUZ12 or EED, although in LNCaP cells the majority of EZH2 coeluted with other PRC2 subunits (Fig. 3A).

We next asked whether the methyltransferase activity of EZH2 is required despite the lack of the other PRC2 components. We replaced the endogenous EZH2 in abl cells with either the wild type (E^{sr}-WT) or two enzymatically inactive mutants [SET domain deletion (E^{sr}-ΔSET) (*11*) and H694A/F672I double point mutation (E^{sr}-DM) (*12*, *13*)] (fig. S10A). Ectopic reexpression of the wild-type EZH2, but not the catalytically inactive mutants, could rescue the effects of EZH2 silencing on both gene activation (Fig. 3B) and androgen-independent growth of CRPC cells (Fig. 3C and fig. S10B). We also found that EZH2 overexpression was sufficient to promote the androgen-independent growth of LNCaP cells and that the enzymatic activity was required (Fig. 3D). Under these conditions, the expression of EZH2-activated target genes was elevated to levels comparable to those in abl cells by wild-type EZH2, but not by the activity-dead mutants (fig. S10C). These results suggest that EZH2 makes use of a PRC2-independent methyltransferase activity for both gene activation and androgen-independent growth.

To determine how EZH2 might be targeted to solo peaks, we conducted motif analysis and found significant enrichment of the androgen receptor (AR) binding motif at EZH2 solo peaks in abl cells (fig. S11A). AR chromatin binding was enriched at the center of EZH2 solo peaks but not at ensemble peaks (fig. S11B). Coimmunoprecipitation detected a robust physical interaction between EZH2 and AR in abl cells (Fig. 3E). The interaction between AR and EZH2 was lost when the endogenous EZH2 was replaced with EZH2 deletion mutants either in Domain I (an N-terminal protein-protein interaction domain) or in the C-terminal SET domain (fig. S12), suggesting the requirement of these two domains for the interaction. EZH2 solo peaks in abl cells significantly overlapped with AR global binding, and EZH2- and AR-activated genes also overlapped significantly in abl cells (fig. S13). However, not all solo peaks contained an AR motif or overlapped with AR binding, which suggests that other factors in addition to AR may contribute to EZH2 recruitment to solo sites.

Although EZH2 depletion did not change AR mRNA or protein levels, it did decrease AR-associated lysine methylation; this required an intact EZH2 enzymatic activity (fig. S14). Such a finding suggests that EZH2 exerts its activation function not by modulating the AR level, but rather through alterations in the methylation of AR or AR-associated proteins. Silencing of EZH2 decreased AR recruitment to solo sites bound by both AR and EZH2 (fig. S15A) and had no significant effects on AR binding to other sites (fig. S15B). Similarly, knockdown of AR decreased EZH2 binding to the colocalized solo peaks (fig. S15C). Depletion of both EZH2 and AR led to a more marked reduction in the expression of co-regulated genes than silencing of either alone (fig. S15D). These results indicate that EZH2 and AR activate a set of target genes through their cooperative recruitment.

To identify what factors determine the functional switch of EZH2 from a repressor to an activator, we examined the phosphorylation status of EZH2, which has been reported to alter its enzymatic activity toward H3K27 (*14*–*17*). Phosphorylation levels at both Ser^{21} and Thr^{492} were elevated in abl cells relative to LNCaP cells, whereas phosphorylation at Thr^{350} was equivalent (Fig. 4A). We replaced the endogenous EZH2 with phosphorylation site mutants to determine the potential role of site-specific phosphorylation in EZH2-mediated gene activation, and found that a Ser^{21} → Ala (S21A) mutant failed to rescue the down-regulation of EZH2-activated genes upon EZH2 silencing in abl cells (fig. S16A). In addition, only the antibody specific for phosphorylated Ser^{21} could detect EZH2 enrichment preferentially at the solo peaks (Fig. 4B and fig. S16B). Furthermore, replacement of endogenous EZH2 by wild-type EZH2 or a phosphomimetic Ser^{21} → Asp (S21D) mutant, but not the S21A mutant, could support the androgen-independent growth of CRPC cells (Fig. 4C and fig. S17, A and B). This same dependence on Ser^{21} phosphorylation was also found for the ability of EZH2 to induce the androgen-independent growth of LNCaP cells (fig. S17C). These results suggest the importance of phosphorylation at Ser^{21} in both EZH2-mediated gene activation and androgen-independent growth. We further confirmed the up-regulation of EZH2 phosphorylation at Ser^{21} in two additional hormone-refractory prostate cancer cell lines, C4-2B and CWR22Rv1 (fig. S18A). Consistent with the report

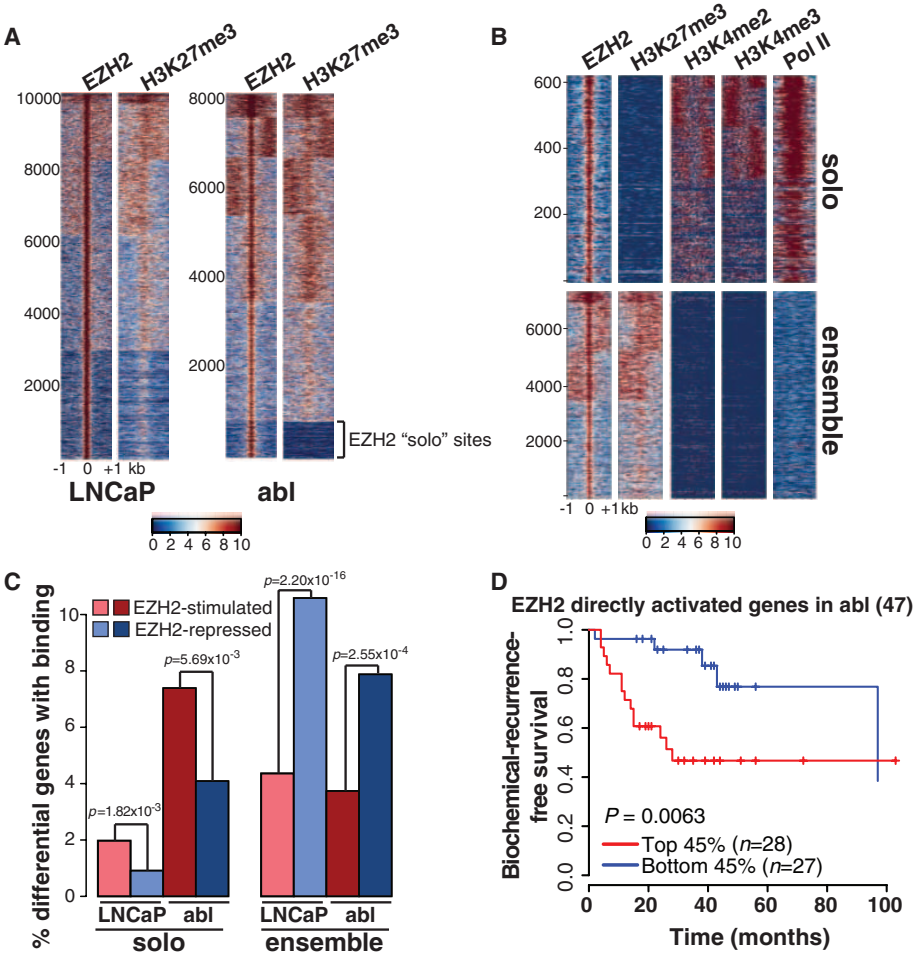

Fig. 2. EZH2 binding without H3K27me3 is associated with gene activation in CRPC. (**A**) Heat maps of EZH2 and H3K27me3 ChIP-seq signal ±1 kb around the EZH2 peak summit in LNCaP and abl cells. The color scale indicates average signal using a 10–base pair window. The numbered index of EZH2 peaks is shown to the left. (**B**) Heat maps of EZH2, H3K27me3, H3K4me2, H3K4me3, and Pol II ChIP-seq signal ±1 kb around EZH2 solo or ensemble peak summit in abl cells. (**C**) Percentages of differentially expressed genes upon EZH2 depletion in LNCaP or abl cells containing EZH2 solo or ensemble peaks within 20 kb around transcription start sites. (**D**) Kaplan-Meier plots of genes directly activated by EZH2 in the Yu *et al.* cohort (*8*). The number of genes is indicated at the top of the plot; *n*, numbers of patients.

that Akt is the kinase for EZH2 phosphorylation at Ser[21] (*16*), we found higher levels of active Akt in abl cells (Fig. 4A). Although both LNCaP and abl cells were PTEN-null, the Akt phosphatase PHLPP-1 (*18*) was decreased in abl cells (fig. S18B), which may contribute to the activation of the phosphatidylinositol 3-kinase (PI3K)–Akt signaling in abl cells. To gain further insight into the involvement of EZH2 phosphorylation in the EZH2 interaction with

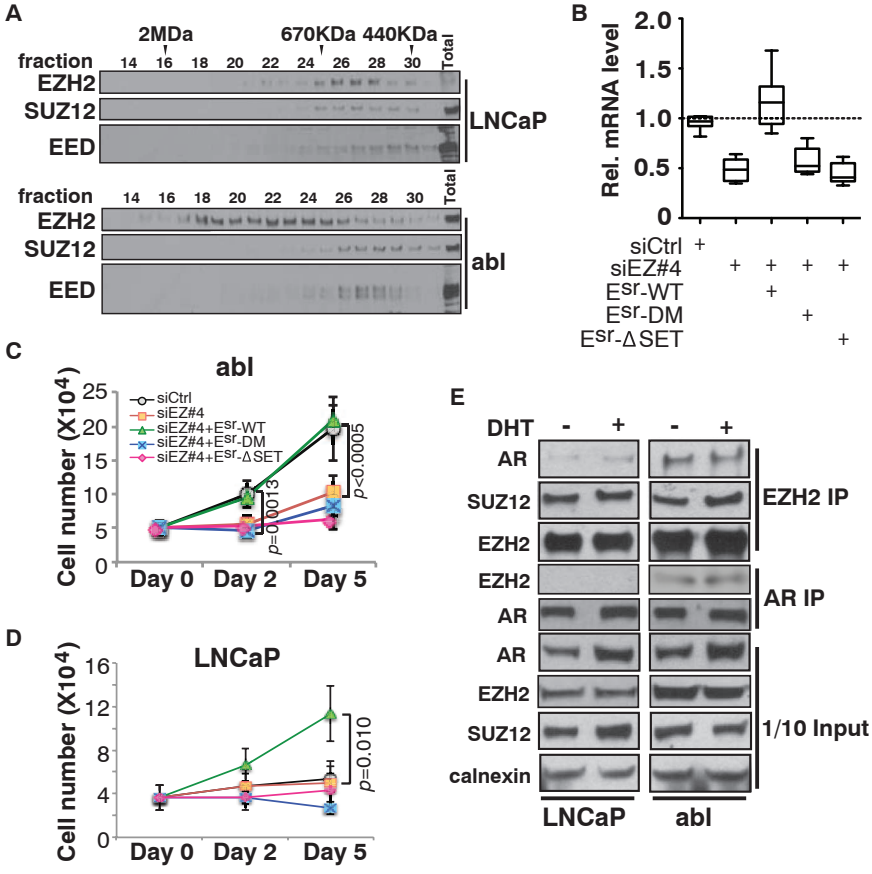

Fig. 3. Requirement of methyltransferase activity and interaction with AR for the EZH2 transactivation function. (**A**) Immunoblot of nuclear extracts from LNCaP and abl cells after gel filtration fractionation. Molecular mass standards are indicated. (**B**) Box plots of minimum to maximum reverse transcription polymerase chain reaction (RT-PCR) values for EZH2-activated genes in abl cells after replacement with wild-type or mutant EZH2 as indicated. (**C** and **D**) Growth of abl cells (C) and LNCaP cells (D) in hormone-depleted medium after replacement with wild-type or mutant EZH2 as indicated. (**E**) Coimmunoprecipitation of EZH2 and AR in LNCaP and abl cells without (–) or with (+) DHT.

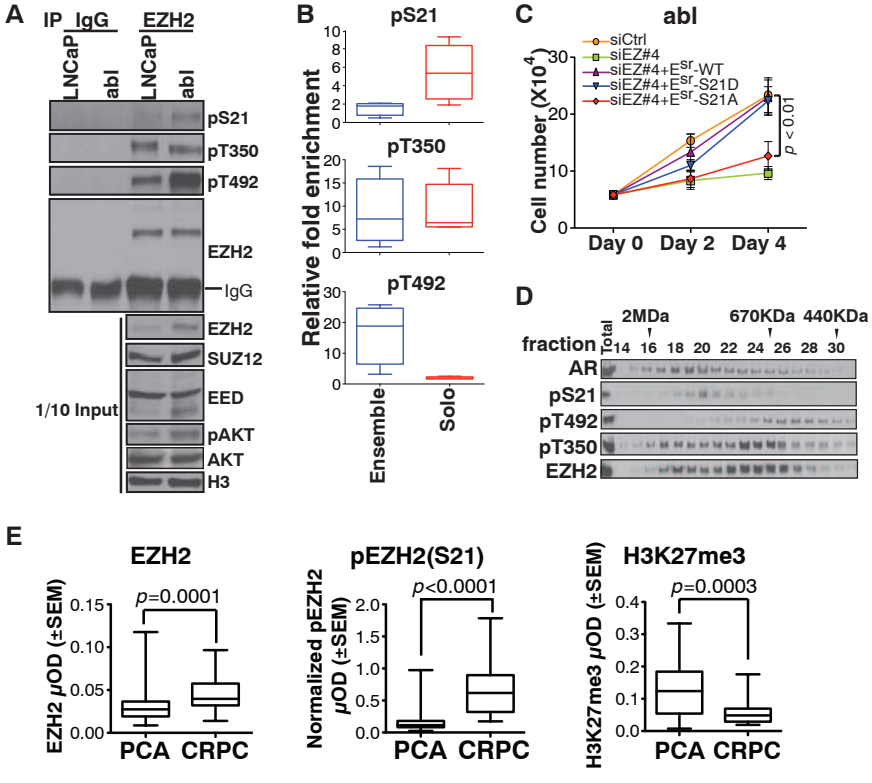

Fig. 4. Critical role of EZH2 phosphorylation at Ser[21] for its functional switch. (**A**) Immunoprecipitation (IP) in LNCaP and abl cells, using control immunoglobulin (IgG) or antibodies specific to EZH2 followed by immunoblotting with the indicated antibodies. (**B**) Box plots of ChIP–quantitative PCR values for EZH2 recruitment to selected ensemble or solo sites, using phosphorylation-specific antibodies. (**C**) Growth of abl cells in androgen-depleted medium after replacement with wild-type or mutant EZH2 as indicated. (**D**) Immunoblot of nuclear extracts from abl cells after gel filtration fractionation. Molecular mass standards are indicated. (**E**) Analysis of EZH2, Ser[21]-phosphorylated EZH2 [pEZH2(S21)], and H3K27me3 protein levels by quantitative immunohistochemistry in neoadjuvant prostate tumors (PCA) and CRPC.

AR, we investigated the copurification of phosphorylated forms of EZH2 and AR by gel filtration and found that pS21 EZH2 predominantly coeluted with AR in a high–molecular weight complex (Fig. 4D). These results suggest a potential role for EZH2 phosphorylation at Ser^{21} to promote its association with an AR-containing complex.

The importance of EZH2 phosphorylation at Ser^{21} in prostate cancer progression was further analyzed by immunohistochemistry in tissue microarrays containing early-stage prostate tumors from a neoadjuvant androgen deprivation therapy trial and metastatic, hormone-refractory tumors (Fig. 4E and fig. S19). As previously reported (*1*), the level of EZH2 in CRPC was higher than during early-stage disease, and pS21 EZH2 was even more significantly increased in CRPC. Intriguingly, H3K27me3 levels significantly decreased with prostate cancer progression, consistent with our observation that the global level of H3K27me3 in abl cells was considerably lower than in LNCaP cells (Fig. 1A). This result further supports our conclusion that the oncogenic activity of EZH2 in CRPC is independent of its Polycomb-repressive function.

This study demonstrates that phosphorylation of EZH2 at Ser^{21}, mediated directly or indirectly by the PI3K-Akt pathway, can switch its function from a Polycomb repressor to a transcriptional coactivator of AR (and potentially other factors). Rescue experiments and the lack of correlation with H3K27me3 levels support a role for EZH2-directed methylation of substrates other than H3K27, including potential nonhistone proteins. The current rationale for EZH2 inhibitor design is based primarily on targeting its Polycomb-repressive activity and uses H3K27me3 as the pharmacodynamic readout (*19*). However, the observed loss-of-function mutations of EZH2 in myelodysplastic syndrome and acute leukemia raise concerns that such inhibitors might exhibit important hematologic side effects (*20*, *21*). Our finding of an altered function for EZH2 in CRPC cells raises the potential to develop inhibitors that specifically target the EZH2 activation function while sparing its PRC2-repressive function. In addition, our finding that EZH2 cooperates with AR-associated complexes and requires phosphorylation to support CRPC growth suggests novel combination therapies for the treatment of metastatic, hormone-refractory prostate cancer (fig. S20).

References and Notes

1. S. Varambally *et al.*, *Nature* **419**, 624 (2002).
2. R. Cao *et al.*, *Science* **298**, 1039 (2002).
3. H. Chen, S. W. Tu, J. T. Hsieh, *J. Biol. Chem.* **280**, 22437 (2005).
4. S. T. Lee *et al.*, *Mol. Cell* **43**, 798 (2011).
5. D. LaJeunesse, A. Shearn, *Development* **122**, 2189 (1996).
6. H. Strutt, G. Cavalli, R. Paro, *EMBO J.* **16**, 3621 (1997).
7. Z. Culig *et al.*, *Br. J. Cancer* **81**, 242 (1999).
8. Y. P. Yu *et al.*, *J. Clin. Oncol.* **22**, 2790 (2004).
9. S. Varambally *et al.*, *Cancer Cell* **8**, 393 (2005).
10. G. V. Glinsky, A. B. Glinskii, A. J. Stephenson, R. M. Hoffman, W. L. Gerald, *J. Clin. Invest.* **113**, 913 (2004).
11. R. Cao, Y. Zhang, *Curr. Opin. Genet. Dev.* **14**, 155 (2004).
12. A. Kuzmichev, K. Nishioka, H. Erdjument-Bromage, P. Tempst, D. Reinberg, *Genes Dev.* **16**, 2893 (2002).
13. P. Joshi *et al.*, *J. Biol. Chem.* **283**, 27757 (2008).
14. S. Chen *et al.*, *Nat. Cell Biol.* **12**, 1108 (2010).
15. S. Kaneko *et al.*, *Genes Dev.* **24**, 2615 (2010).
16. T. L. Cha *et al.*, *Science* **310**, 306 (2005).
17. Y. Wei *et al.*, *Nat. Cell Biol.* **13**, 87 (2011).
18. T. Gao, F. Furnari, A. C. Newton, *Mol. Cell* **18**, 13 (2005).
19. A. Sparmann, M. van Lohuizen, *Nat. Rev. Cancer* **6**, 846 (2006).
20. G. Nikoloski *et al.*, *Nat. Genet.* **42**, 665 (2010).
21. P. Ntziachristos *et al.*, *Nat. Med.* **18**, 298 (2012).
22. A. Kuzmichev, T. Jenuwein, P. Tempst, D. Reinberg, *Mol. Cell* **14**, 183 (2004).

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Supplementary Materials

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Airn Transcriptional Overlap, But Not Its lncRNA Products, Induces Imprinted *Igf2r* Silencing

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Mammalian imprinted genes often cluster with long noncoding (lnc) RNAs. Three lncRNAs that induce parental-specific silencing show hallmarks indicating that their transcription is more important than their product. To test whether *Airn* transcription or product silences the *Igf2r* gene, we shortened the endogenous lncRNA to different lengths. The results excluded a role for spliced and unspliced *Airn* lncRNA products and for *Airn* nuclear size and location in silencing *Igf2r*. Instead, silencing only required *Airn* transcriptional overlap of the *Igf2r* promoter, which interferes with RNA polymerase II recruitment in the absence of repressive chromatin. Such a repressor function for lncRNA transcriptional overlap reveals a gene silencing mechanism that may be widespread in the mammalian genome, given the abundance of lncRNA transcripts.

Macro long noncoding (lnc) RNAs such as *Airn* (*1*), *Kcnq1ot1* (*2*), or *Nespas* (*3*) that silence imprinted gene clusters offer important epigenetic models for the numerous lncRNAs mapped in the mammalian genome (*4*–*6*). In the *Igf2r* imprinted cluster, the paternally expressed *Airn* (antisense *Igf2r* RNA noncoding) macro lncRNA silences in cis the paternal alleles of *Igf2r*, *Slc22a3*, and *Slc22a2* (*1*). *Airn* may use different silencing mechanisms, because *Igf2r* is silenced in all embryonic, extraembryonic, and adult tissues that express *Airn*, whereas *Slc22a2* and *Slc22a3* are only silenced in some extraembryonic lineages (*7*, *8*). In support of this, *Slc22a3* silencing in the placenta depends on the *Airn* lncRNA product recruiting EHMT2 histone methyltransferase, whereas *Igf2r* silencing does not (*9*). *Igf2r* silencing is also not dependent on Polycomb-group proteins or DNA methylation (*10*, *11*). Thus, the mechanism by which *Airn* silences *Igf2r*, the only gene in this cluster with an essential embryonic function (*12*), remains unknown. *Airn* transcription overlaps the *Igf2r* promoter but not the *Slc22a3* or *Slc22a2* promoters (fig. S1A), indicating that silencing could depend on *Airn* transcriptional overlap independent of the *Airn* lncRNA product.

To test the role of *Airn* transcription versus product in *Igf2r* silencing, we used homologous recombination in embryonic stem (ES) cells to insert polyadenylation (polyA) cassettes on the paternal chromosome that truncate *Airn* to different

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Science

EZH2 Oncogenic Activity in Castration-Resistant Prostate Cancer Cells Is Polycomb-Independent

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Alternative Role for EZH2

Epigenetic regulators are implicated in cancer progression and proposed as therapeutic targets. **Xu *et al.*** (p. 1465; see the Perspective by **Cavalli**) report that EZH2 (Enhancer of zeste homolog 2), a factor previously thought to exert its oncogenic function primarily as part of the polycomb repressive complex, acts through a distinct mechanism in cells of castration-resistant prostate cancer. Rather than exclusively silencing gene expression through histone methylation, EZH2 acts as a transcriptional coactivator. The activation function of EZH2 plays a critical role in the growth of castration-resistant prostate cancer cells, which could be relevant in future drug development.