Liver X receptors constrain tumor development and metastasis dissemination in PTEN-deficient prostate cancer

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Advanced prostate cancer (PCa) is a clinical challenge as no curative therapeutic is available. In this context, a better understanding of metastasis and resistance mechanisms in PCa is an important issue. As phosphatase and tensin homolog (PTEN) loss is the most common genetic lesion in such cancer, we investigate human data sets for mechanisms that can constrain cancer evolution in this setting. Here we report a liver X receptor (LXR) signature, which tightly correlates with PTEN loss, in PCa. Accordingly, the LXR pathway is deregulated in prostate carcinomas in Pten-null mice. Genetic ablation of LXRs in Pten-null mice, exacerbates PCa invasiveness and metastatic dissemination, which involves mesenchymal transition and accumulation of matrix metalloproteinases. Mechanistically, PTEN deletion governed LXR transcriptional activity through deregulation of cholesterol de novo synthesis, resulting in accumulation of endogenous LXR ligands. Our study therefore reveals a functional circuit linking PTEN and LXR, and highlights LXRs as metabolic gatekeepers that are able to constrain PCa progression.
**Results**

**LXRs are gatekeepers of Pten-null prostate tumor progression.**

We have recently reported that LXRs attenuate prostate intraepithelial neoplasia (PIN) in vivo. To evaluate the status of the LXR-controlled transcriptional program in advanced prostate tumors we compared the expression of a canonical set of LXR target genes by analyzing public datasets of human PCa collections. ONCOMINE analyses identified upregulation of the LXR transcriptional program, which was most prominent in metastatic PCAs (Figs. 1a, b and Supplementary Fig. 1A). Interestingly, evaluation of enrichment profiling (Gene Set Enrichment Analysis, GSEA) of gene sets sensitive to PTEN loss supported the existence of an inverse correlation between expression of LXR target genes and PTEN status in humans (Fig. 1c and Supplementary Fig. 1B). We therefore decided to evaluate LXR signaling in prostate samples derived from control (Pten+/+Lxrαβ−/−) and prostate-specific Pten-null (Pten−/−Lxrαβ−/−) mice. Consistent with the finding in the human data set, levels of the established LXR targets ABCA1, FAS and APOE were substantially increased in prostate from Pten−/−Lxrαβ−/− mice (Fig. 1d, e). Importantly, we confirmed that dysregulated LXR signaling in these mice was LXR dependent. Indeed, expression of the three LXR target genes decreased in Pten−/−Lxrαβ−/− mice (Fig. 1e and Supplementary Fig. 2A, B) generated by mating of Pten−/− with Lxrαβ−/− mice. The effect of PTEN loss on LXR signaling was not limited to ABCA1, FASN and APOE, as other LXR-regulated genes also displayed aberrant expression (Fig. 1f and Supplementary Fig. 2C, D). Furthermore, these changes do not represent global deregulation of metabolic gene expression, as a panel of other metabolic genes remain unchanged (Fig. 1g), and is also not the result of alterations in the expression level of Lxrα or Lxrβ in mouse prostate (Fig. 1h).

To investigate the role had by LXRs in prostate carcinogenesis, we studied the consequence of their deletion in Pten−/− mice. Macroscopic analyses of prostata revealed that loss of Lxars resulted in a marked increase in gland size and weight (Fig. 2a, b). Importantly, Lxrαβ−/− control littermates had normal prostates, demonstrating that the loss of Lxars is not intrinsically carcinogenic (Supplementary Fig. 3A). Consistent with the prostate phenotype, comparison of prostates from Pten−/− and Pten−/−Lxrαβ−/− mice revealed a significant increase in the frequency of invasive carcinoma both in 2- and 6-month-old mice (Fig. 2c, d and Supplementary Fig. 4A–D). Enhanced tumor invasiveness in Pten−/−Lxrαβ−/− mice was further confirmed using smooth muscle actin breakdown staining, as a proxy for tumor barriability (Fig. 2c and Supplementary Fig. 4E, F). Proliferation, as assessed by Ki67 scoring, was increased in prostate from Pten−/−Lxrαβ−/− mice (Fig. 2f, g and Supplementary Fig. 5A–D), revealing a marked increase in gland size and weight (Fig. 2i, j). Remarkably, histopathological analysis of distant organs showed a high frequency of metastatic spread (Fig. 2i and Supplementary Fig. 5E), which was confirmed by the presence of CK18- and PSCA-positive nodules in Pten−/−Lxrαβ−/− mouse lungs and lymph nodes (Fig. 2k). We observed metastatic lesions in some 2-month-old Pten−/−Lxrαβ−/− mice that progressed to severe and systematic dissemination in 6-month-old animals (Fig. 2i and Supplementary Fig. 5F and 5G). These observations stand in stark contrast with our observation in Pten−/−Lxr−/− mice, which rarely developed metastasis before 1 year of age. Taken together, these results provide strong in vivo evidence that LXRs constrain cancer progression and dissemination in Pten−/−Lxr−/− mice.

**PTEN deletion acts on LXR through PI3K/AKT.** To investigate the mechanism linking PTEN and LXRs, we took advantage of previously established immortalized cells derived from mouse prostate, mouse prostate epithelial cells (MPECs) (Supplementary Fig. 6A, B). As observed in vivo, expression of LXR target genes was increased in the absence of PTEN in MPECs (Fig. 3a, b and Supplementary Fig. 6C), establishing a functional link between PTEN deletion and LXR activity in tumor epithelial cells. To extend this observation to a relevant human system, we investigated LNCaP and PC3 cells, two established PTEN-negative cell lines commonly used to study PCa. First, we used LNCaP cells that have been genetically engineered to express a doxycycline-inducible PTEN–green fluorescent protein (GFP) fusion protein (LNCaP-PTEN). Remarkably, doxycycline induction leads to a marked decrease in ABCA1, APOE and FASN proteins in these cells (Fig. 3c, d and Supplementary Fig. 6D). This effect was not limited to ABCA1 as additional LXR-regulated genes responded similarly (Fig. 3e). To test whether the...
**Fig. 1** PTEN-loss carcinogenesis is associated with transcriptional upregulation of LXR target genes. **a** ONCOMINE boxed plot of PTEN, FASN and APOE expression levels between human prostate gland, prostate carcinoma and metastasis in various human data sets. **b** Heatmap of LXR target gene signature and PTEN expression in Grasso et al. data set. **c** Gene Set Enrichment Analysis of LXR target gene signature based on PTEN status using Grasso et al. data set. **d** HE staining and immunofluorescence against PTEN, AKT S473 and ABCA1 of wild type (WT) and Pten<sup>−/−</sup> mouse prostate tissues from 6-month-old animals. Scale bar, 100 µm. Nuclei are stained using Hoescht (blue). **e** Western blot analysis of PTEN, AKT S473, AKT, FASN, APOE and GAPDH of wild type (WT), Pten<sup>−/−</sup> and Pten<sup>−/−</sup> Lxrαβ<sup>−/−</sup> mouse prostatic tissues from 6-month-old animals. **f-h** Relative expression of LXR targets genes, non-LXR target gene panel, Lxrα (Nr1h3) and Lxrβ (Nr1h2). All data are represented as mean ± SEM and statistical analyses were performed with the Student’s t-test; *p < 0.05, **p < 0.01 and ***p < 0.001. See also Supplementary Figs. 1 and 2.
effect of PTEN on LXR signaling was dependent on its intrinsic phosphatase activity we transfected LNCaP cells with expression constructs encoding either wild type (WT) or catalytically inactive PTEN (PTEN C124A). Whereas expression of WT PTEN decreased LXR-regulated gene expression, the catalytic mutant failed to do so (Fig. 3f and Supplementary Fig. 6E). Accordingly, transfection of mutant PTEN did not reduce the level of APOE and FASN protein in these cells (Fig. 3g). Similar results were obtained using PC3 cells, a second PTEN-negative cell line (Supplementary Fig. 6F–H). Collectively, these results demonstrate that PTEN’s intrinsic phosphatase activity is an important determinant of LXR signaling. As PTEN is a key regulator of PI3K/AKT signaling, we reasoned that this signaling pathway may couple PTEN and LXRs. Consistent with this idea, LNCaP
and PC3 cells treated with two PI3K inhibitors, Wortmannin and LY294002, exhibited decreased expression of LXR target genes (Fig. 3h, i and Supplementary Fig. 7A–D). To further substantiate the role of PI3K/AKT in regulating LXR signaling, we made use of a dominant negative AKT construct. Similar to the PI3K inhibitors, this genetic manipulation decreased expression of LXR target genes in both cell lines (Fig. 3i and Supplementary Fig. 7E–G). Reciprocally, we reasoned that forced activation of PI3K/AKT-dependent signaling in PTEN-positive cells should increase LXR pathway. To test this notion, we expressed a constitutively active form of AKT (MyrAKT) or of the PI3K catalytic subunit p110 (p110CAAX) in PTEN-positive DU145 cells. As posited, this resulted in a substantial increase in LXR signaling (Fig. 3k, l and Supplementary Fig. 7H). Therefore, our pharmacologic and genetic experiments substantiate a key role of the PTEN/PI3K/AKT axis in regulating LXR signaling in prostate epithelial cells.

To establish that regulation of LXR signaling by PTEN involves modulation of their transcriptional activity we introduced an LXXR-driven reporter construct in Pten–/– mouse embryonic fibroblasts (MEFs). In line with our earlier observations in the prostate-derived cell lines, PTEN expression decreased the LXR-reporter signal in an activity-dependent manner, as this was not observed when a Pten C124A mutant was introduced (Fig. 3m and Supplementary Fig. 7I). We therefore conclude that PTEN loss induces expression of LXR target genes by regulating LXR’s transcriptional activity.

PTEN deletion drives LXR activation through cholesterol. We next sought to determine how the PTEN/PI3K/AKT axis modulates LXR activity in PCa. Based on our experiments with the synthetic LXR ligand we postulated that this might be mediated by the production of endogenous LXR ligands (e.g. oxysterols) and tested this by determining the hydroxysterol species present in prostate of WT and Pten–/– mice focusing on established LXR ligands. We observed that levels of 5,6β-epoxy-cholesterol and 5,6α-epoxy-cholesterol, but not 22(R)-hydroxycholesterol and 27-hydroxycholesterol (27-OHC) were significantly increased in Pten–/– prostate (Fig. 4a and Supplementary Fig. 8A). Reciprocally, transfection of PTEN or an AKTd/n expression constructs into PC3 PTEN-negative cells significantly decreased 5,6β-epoxy-cholesterol and 5,6α-epoxy-cholesterol accumulation (Fig. 4b and Supplementary Fig. 8B). Similarly, accumulation of 22(R)-hydroxycholesterol and 27-hydroxycholesterol was also slightly reduced as a result of this treatment. Together, these findings pointed out 5,6α-epoxy-cholesterol as a ‘‘negative’’ endogenous LXR ligand in mouse prostates as previously reported by Berrodin et al. in keratinocytes. In order to determine that PTEN governs the production of endogenous LXR ligands, we induced PTEN expression in LNCAP doxycycline-inducible cells. Consistent with reduced production of endogenous ligands, the level of ABCA1 protein decreased (Fig. 4c). However, after treating PTEN-induced cells with T0901317, the level of Abca1, as well as others LXR target genes was fully restored (Figs. 4d, 5). This corroborates data from MPECs and showed that intrinsic LXR signaling was abolished in PTEN positive cells, but that ligand availability may be the limiting factor. Consistent with this, T0901317 also abolished LXR expression by P13K inhibitors in LNCAp and PC3 cells (Supplementary Fig. 8C, D).

To definitively demonstrate that PTEN status governs production of oxysterols, we tested whether PC3 culture supernatants (i.e., conditioned medium) contain LXR agonists, by determining whether the supernatants can induce expression of a an LXR reporter system (Gal4-LXRβ and PDE4b-/-/KrasG12D) reconstituted in MEFs (Fig. 4e). We found that supernatants from PC3 cells transduced with a virus encoding SULT2B1b, a LXR agonist-inactivating enzyme20–22 resulted in a marked decrease of LXR transcriptional activity monitored either using the reporter assay (Fig. 4e and Supplementary Fig. 8E) or when directly measuring target gene expression (Fig. 4f). Similarly, PTEN or AKTd/n transfected PC3 cells exhibited lower induction of LXR and LXRβ transcriptional activity (Fig. 4g). These experiments

Fig. 2. Cytobalan. Pten-mutant mice leads to cancer progression and metastasis dissemination. a Gross anatomy of representative prostates at 6 months of age. Seminal vesicles (SV), bladder (B), anterior prostate (AP), dorsal-lateral prostate (DLP) and ventral lobe (VP). Scale bar, 1 cm. b Relative weight of prostates wild type (WT), Pten+/– and Pten–/–/KrasG12D (N = 16/7/14). Statistical analyses were performed with the Student’s t-test. c Stained sections of representative dorsal prostate (DP) at 8, 11 and 20 weeks. Scale bar, 100 µm. d Histological evaluation of dorsal lobe lesions, Low-grade PIN, high-grade PIN, in situ carcinoma or invasive carcinoma. Three distant sections from each mouse (10 mice per group) were scored (p2 = 17.27; p = 0.0006). e Ki67 and SMA (smooth muscle actin) immunofluorescence performed on Pten+/–- and Pten–/–/KrasG12D prostate specimens. Acini breaks have been quantified using following criteria: discontinuous SMA staining and presence of Ki67-positive staining in surrounding stromal compartment. Nuclei are stained using Hoescht (blue). Scale bar 100 µm. (N = 10 per group). f Representative Ki67 immunohistochemistry on prostatic tissues from each genotype, scale bar 100 µm. g Quantification of Ki67-positive staining (N = 8 per group). h Kaplan–Meier cumulative survival analysis showing significant decrease (p < 0.0001) in lifespan in the Pten+/–-/KrasG12D compared with Pten+/–/- group. (N = 10/11/12). i Recaptitive table of metastatic phenotypes in Pten+/– and Pten–/–/KrasG12D animals. j Gross anatomy of lungs from Pten+/– vs. Pten–/–/KrasG12D from 6-month-old mice. k HE-stained sections and immunofluorescence detection of primary tumor site (prostate), lumbar lymph nodes and lung of Pten+/– and Pten–/–/KrasG12D using specific (CK18/PSCA) prostate markers. High-magnification depicted cell arrangement within the host tissue. Scale bar, 100 µm. Nuclei are stained using Hoescht (blue). All data are represented as mean ± SEM. ***p < 0.001. See also Supplementary Figs. 3, 4 and 5.
support the notion that PI3K activity is associated with the production of endogenous LXR agonists, and that therefore an important consequence of PTEN loss is enhanced LXR signaling. Oxysterols originate from both enzymatic activity and lipoperoxidation, and we therefore aimed to identify the source of the elevated LXR ligands produced as a result of PTEN loss. Analysis of PTEN-null prostates revealed increased expression of Cyp46 and Cyp7a1, yet the level of the corresponding products of these enzymes was not elevated (Supplementary Fig. 9A). This finding indicate that 5,6-epoxy-cholesterol could be produced from cholesterol by an unidentified cytochrome P450. Such stereospecific transformations have already been reported in the microsomal fraction of the bovine adrenal cortex24. To evaluate the role of lipoperoxidation we tested whether vitamin E, a potent
inhibitor of this process that also blocks cholesterol epoxidation, altered the PTEN-dependent LXR response. This may be particularly relevant, as 5,6-epoxy-cholesterol and 5,6α-epoxy-cholesterol represent major accumulating sterol species in LNCaP cells transfected with AKTd/n (dominant-negative) expression vector. MEFF choleseral cholesterol represent major accumulating sterol species in particular relevant, as 5,6α-epoxy-cholesterol and 5,6δ-epoxy-cholesterol altered the PTEN-dependent LXR response. This may be altered. Our results suggest that lipoperoxidation participate, at least in part, to LXR's activation when PTEN is lost.

Another source of increased oxysterols and LXR ligands in Ptenf−/− prostate may be a result of increased de novo cholesterol synthesis, as previously proposed in human-derived prostate cell lines. To test this hypothesis, we determined the expression of genes in the SREBP2-regulated cholesterol synthesis pathway. Our analysis revealed a marked increase in expression of SREBP2-regulated genes and of SREBP2 itself (Fig. 5a). Furthermore, the levels of cholesterol biosynthesis intermediates and cholesterol were also elevated (Fig. 5b). Notably, we also detected marked elevation of desmosterol, a potent endogenous LXR ligand. We next reasoned that if enhanced cholesterol synthesis drove LXR signaling in Pten-null tumors, this process should be sensitive to inhibition by statins that block HMGCRA reductase, a rate-limiting step in cholesterol biosynthetic pathway. As expected, simvastatin treatment increased expression of HMGCRA in LNCaP cells (Fig. 5c) and reciprocally repressed both ABCA1 and FASN expression in LNCaP cells (Fig. 5c and Supplementary Fig. 11A), as well as in PC3 cells (Supplementary Fig. 11B) and MPECs (Supplementary Fig. 11C). Repression of LXR pathway by statins could be completely overcome by T0901317 treatment (Fig. 5c and Supplementary Fig. 11A–C), consistent with the idea that de novo synthesis was responsible for the production of endogenous LXR ligands in response to PTEN loss. To conclusively demonstrate that reduced endogenous LXR ligands production in PTEN-null tumors was the underlying cause for decreased LXR signaling following statin treatment, we blocked HMGCRA with simvastatin and supplemented cultured cells with a high concentration of mevalonate, the product of HMGCRA activity. As expected, mevalonate counteracted inhibition of de novo synthesis by simvastatin, restored metabolic pathway and expression of ABCA1 (Fig. 5d). Restoration of ABCA1 and FASN expression by exogenous mevalonate was dose-dependent (Fig. 5e) and was mirrored by a corresponding decline in HMGCRA expression. Thus, having established that altered production of endogenous ligands underlies the effect of PTEN on LXRs, we reasoned that SREBP2, the master transcriptional regulator of cholesterol synthesis, is sensitive to the PTEN status. To evaluate activity of the SREBP2-regulated pathway we monitored the processing of SREBP2 into the transcriptional active "mature" form and the levels of the SREBP2 canonical targets, HMGCRA, and SQLE. Pharmacological inhibition of PI3K in LNCaP cells markedly decreased SREBP2 processing and the protein levels of HMGCRA and SQLE (Fig. 5f). Similar results were obtained in PC3 cells using PI3K inhibitors (Supplementary Fig. 11D) and by overexpression of a dominant-negative AKT expression construct (Supplementary Fig. 11E). Interestingly, restoration of de novo synthesis by exogenous mevalonate in PC3 cells in the context of PI3K inhibition increased ABCA1 but also the levels of APOE, another LXR target (Fig. 5g). Finally, SREBP2 knockdown in PC3 cells resulted in a significant decrease of SREBP2 and LXR target genes expression (Fig. 5h) and in the ability of PC3 supernatants to induce LXR transcriptional activity (Supplementary Fig. 11F).

Cumulatively, these experiments support the idea that the PI3K pathway controls LXR signaling by controlling production of endogenous ligand through cholesterol de novo synthesis. To test this idea in vivo, we orally administered simvastatin to Ptenf−/− mice. This treatment suppressed accumulation of both Fasn and Abca1 at the messenger RNA and protein level (Figs. 5i, j), which was not observed in MPECs derived from Ptenf−/−/Lxrαf−/− prostate (Supplementary Fig. 11C). Simvastatin treatment not only blocks LXR activation in the absence of PTEN but also decreased cholesterol supply from de novo synthesis. Consistent with the role of cholesterol in tumor growth, simvastatin treatment was associated with a decrease in tumor progression and proliferation in Ptenf−/− mice (Supplementary Fig. 12A–E). Therefore, PTEN inactivation in PCa cells results in increased cholesterol de novo synthesis, which results in increased production of endogenous LXR ligands and altered sterol balance in tumor tissue.

LXRs control tumor invasiveness and metastatic spread. The dramatic growth of Ptenf−/− adenocarcinomas when Lxrs were genetically ablated, and particularly the high penetrance of metastatic disease in this model, prompted evaluation of the role of LXRs in the control of carcinogenic invasiveness. In order to identify the underlying molecular mechanisms, we conducted a transcriptomic analysis of WT, Lxrαf−/−, Ptenf−/− and Ptenf−/−/Lxrαf−/− prostate samples (Supplementary Fig. 13A–C). GSEA analysis of transcriptomic data identified epithelial-mesenchymal transition (EMT) as the most deregulated pathway in Ptenf−/−/Lxrαf−/− compared with Ptenf−/− prostates, consistent with the high occurrence of metastasis following inactivation of both Pten and Lxrs. Further analysis of the EMT markers Zeb1, Twist1, Twist2, Snai1 and Vimentin between

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Fig. 3 PTEN controls LXR activities in PCa cells through PI3K/AKT pathway. a Relative expression levels of Abca1, Abcg1, Idol, Fasn and (b) PTEN, AKTS473, AKT, PTEN, ABCA1 and β-ACTIN protein accumulation levels in MPECs (mouse prostatic epithelial cells) wild-type (+/+) and Ptenf−/−, c Immunofluorescence of PTEN-GFP and ABCA1 in LNCaP-PTEN inducible cell line treated with DMSO or Doxycycline (25 μM). Nuclei are stained using Hoechst (blue). Scale bar: 100 μm. d Quantitative measurement of ABCA1-positive cells has been performed. e Relative expression of LXR target genes in LNCaP-PTEN inducible cell line treated with DMSO or Doxycycline (25 μM). f Relative gene expression analysis and protein accumulation of LXR targets in LNCaP cells transfected with PTEN and PTENC124A expression construct vs. empty vector. h Relative expression of LXR target genes in LNCaP cells transfected with Pten inhibitors Wortmannin (0.5 μM) or LY294002 (20 μM). i Relative accumulation of ABCA1, ABCG1, FASN and IDOL in LNCaP cells transfected with MyrAKT or MyrAKT and p110CAAX (dominant-positive) expression vector. j Immunofluorescence against AKTS473 and ABCA1 in DU145 transfected with myrAKT or p110CAAX (dominant-positive) expression vector. Nuclei are stained using Hoechst (blue). Scale bar: 100 μm. Relative accumulation of ABCA1, ABCG1, FASN and IDOL in DU145 transfected with myrAKT of p110CAAX expression vectors. k Luciferase activity measurement in MEF Ptenf−/− transfected with 3xLXRE-Luc reporter construct or tk-Luc construct as a control and with PTEN and PTENC124A expression construct versus empty vector. l Luciferase activity measurement in MEF/F+C, Lxrαf−/−, Lxrβf−/− or Lxrαf−/−/Lxrβf−/− transfected with 3xLXRE-Luc reporter construct and expression vector encoding myrAKT. T0901317 (1 μM) treatment has been performed as a control. MEF Lxrαf−/− were rescued using LXRα and LXRβ expression vectors. m Abca1 and Fasn relative expression in MPEC Ptenf−/− and Ptenf−/−/Lxrαf−/− treated with Wortmannin (0.5 μM), LY294002 (20 μM) and/or T0901317 (1 μM). For whole experiments, the results represent the means ± SEM of three independent experiments; *p < 0.05; **p < 0.01; ***p < 0.001. See also Supplementary Figs 6 and 7.
revealed that loss of Lxrs in the context of Pten inactivation resulted in enhanced expression of these markers, consistent with a switch from epithelial to mesenchymal identity (Figs. 6a-c). Further consistent with the EMT phenotypic shift, the Ncad/Ecad ratio was inverted in Pten<sup>−/−</sup>Lxra<sup>−/−</sup> prostate tumors (Figs. 6a, b). Another well-established determinant that supports tumor aggressiveness is the overexpression of matrix metalloproteinases (MMPs), which allow cells to efficiently degrade the surrounding...
Fig. 5 PTEN deficient results in an increased cholesterol de novo synthesis. a Relative accumulation of Srebp2, Hmgcr, Sqs and Hmgcs1 transcripts in prostate of wild type (WT) and Ptenpc−/− mice. (N = 10 per group). b Accumulation of cholesterol precursors, lanosterol, lathosterol, desmosterol and cholesterol in prostate samples from wild type (WT) (N = 4) and Ptenpc−/− mice (N = 5). c qPCR analysis of HMGCR, ABCA1 and FASN expression in LNCaP cells treated with simvastatin alone (2,5 µM) alone or with T0901317 supplementation (1 µM). Experiments have been performed in three experimental replicates. d qPCR analysis of ABCA1 and HMGCR expression in LNCaP cells treated with Simvastatin (2,5 µM) alone or with mevalonate (500 µM). e qPCR analysis of HMGCR, ABCA1 and FASN expression in PC3 cells treated with LY294002 (20 µM) alone or with increasing amounts of mevalonate (100 µM, 500 µM, 1 mM, 10 mM). f Western blot analysis of SREBP2 cleaved form, HMGCR and SQLE respective accumulation in LNCaP cell line. AKTS473 is used to confirm Wortmannin and LY294002 treatment efficiency and β-ACTIN as a loading control. g Western blot analysis of HMGCR, SQLE and APOE in PC3 cells treated with LY294002 (20 µM) alone or in combination with mevalonate (500 µM). AKTS473 and AKT are used to confirm Wortmannin and LY294002 treatment efficiency and β-ACTIN as a loading control. h SREBP2 and LXR target genes expression in PC3 cells transfected with siSREBP2 or siGFP as control. Experiments have been performed in three experimental replicates. i Relative expression of Hmgcr, Abca1 and Fasn on prostate samples from wild type mice and Ptenpc−/− mice treated with vehicle (methylcellulose) or Simvastatin (40 mg kg−1). j Immunofluorescence detection of FASN and ABCA1 on prostate samples from wild type mice and Ptenpc−/− mice treated with vehicle (methylcellulose, N = 6) or Simvastatin (40 mg kg−1, N = 6). Nuclei are stained using Hoescht (blue), scale bar 100 µm. k qPCR analysis of Abca1 and Fasn expression on MPEC Pten−/− or Pten−/− lnxβ−/− treated with simvastatin alone (2.5 µM) or in combination with T0901317 (1 µM), N = 3 per group. All data are represented as mean ± SEM and statistical analyses were performed with the Student’s t-test. *p < 0.05; **p < 0.01; ***p < 0.001. See also Supplementary Figs. 11 and 12
matrix and migrate. Accordingly, we found enhanced expression of *Mmp1*, *Mmp2*, *Mmp7* and *Mmp9* (Fig. 6d), and significant accumulation of MMP9 protein in *Pten*−/−Lxraβ/−/− prostates (Fig. 6e). In contrast, expression of *Timp2*, an endogenous inhibitor of MMPs, was decreased (Fig. 6d). Furthermore, immunostaining of metastasis in lungs of *Pten*−/−Lxraβ/−/− revealed extensive MMP9 signal at the interface between metastatic cells and the pulmonary matrix (Fig. 6f). This demonstrates the invasive properties of metastatic cells within the lung.

Our results clearly demonstrate that LXRs can constrain PCa metastatic spread in vivo. However, in our model LXRs are inactivated in all cells of the body. Therefore, metastatic spread could be the result of LXRs inactivation within epithelial cells, their microenvironment or both. To investigate the epithelial cell-autonomous effects of *Lxr* inactivation, we first tested the ability of PC3 cells transduced with SULT2B1 to migrate across a matrix, using Boyden chambers (Fig. 7a). Consistent with a direct role of LXRs within epithelial cells, PC3 cells that overexpressed SULT2B1 showed a greater capacity to migrate throughout the matrix. In aggregate with our earlier results, this suggests that oxysterol production, and as a result LXR activation, act to limit PC3 invasiveness in vitro. To address whether this is also the case in vivo, we could not use the *Pten*−/−Lxraβ/−/−, as these harbor a global loss of LXR that does not allow the isolated study of prostate cells. As an alternative, we therefore evaluated metastatic dissemination in immune-deficient mice that were implanted with prostate tumors originating from *Pten*−/− and *Pten*−/−Lxraβ/−/− (Fig. 7b). Consistent with data in the genetic models, *Pten*−/−Lxraβ/−/− implants exhibited a higher proliferation rate than *Pten*−/− implants (Figs. 7c, d). Importantly, metastatic dissemination to peripheral organs was only observed in mice implanted with *Pten*−/−Lxraβ/−/− tumors with a 100% penetrance (Figs. 7e, f). Therefore, our results strongly support the idea that the tumor-suppressive activity of LXRs in PCa is cell autonomous and is the result of altered cellular cholesterol metabolism.

Discussion

Management of advanced PCas requires a better understanding of the molecular mechanisms influencing development, progression and eventual metastasis in order to identify tractable therapeutic targets. The most important aspect of our study is the finding of a functional interaction between *PTEN* and LXRs. Specifically, we demonstrate that *PTEN* loss, the most frequent genetic alteration observed in human PCAs, is accompanied with increased activation of the LXR transcriptional pathway. Accordingly, combined ablation of both *Lxr* isoforms and *Pten* in a mouse prostate led to a dramatic increase in prostate carcinoma invasiveness and associated metastatic dissemination. These findings highlight the crucial protective role of LXRs during prostate carcinogenesis. The molecular mechanisms sustaining this deregulation involves enhanced activity of the PI3K/AKT pathway. Altered signaling of this pathway leads to aberrant LXR activation as a result of increased production and accumulation of endogenous ligand and, in turn, this, inhibition of the mevalonate pathway by blocking de novo cholesterol synthesis decreases LXR target gene expression and reduces tumor aggressiveness in vivo. Taken together, our study identifies LXRs as an important determinant of PCa in the setting of *PTEN* loss owing to their ability to suppress tumor invasiveness and dissemination.

Altered cholesterol metabolism is emerging as a metabolic signature of cancer cells. In 1942, accumulation of cholesterol was already reported in adenomas of enlarged prostates. This finding has been overlooked for a long time and viewed as a secondary metabolic adaptation of PCa tumors. This point of view has recently been challenged by several studies and, notably, accumulation of cholesterol esters in lipid droplets induced by *PTEN* loss is now recognized as a hallmark of PCa aggressiveness. Accordingly, manipulations leading to the depletion of cholesterol-ester storage result in reduced PCa invasiveness, highlighting the importance of cellular mobilization of cholesterol as a powerful driver of tumor aggressiveness. In line with this idea, metabolomic analysis of PCa metastasis revealed significant accumulation of cholesterol that was also associated with high levels of the LDLR. Together with our data, these findings emphasize the association between cholesterol accumulation and metastatic processes, as we are also reporting here. The molecular mechanisms governing tumor cholesterol homeostasis in PCa are, however, less clear. The autonomous capability of cells to ensure cholesterol de novo synthesis in response to *PTEN* loss warrants re-evaluation of the classical paradigm of cholesterol homeostasis regulation. Indeed, PCa cells exhibit an increase in both expression and cleavage of SREBP2, a transcription factor that controls the cholesterologenic program, with a concomitant accumulation of hydroxycholesterol species. Studies conducted on LNCaP and PC3 cells lines showed that sterol sensing and the cholesterol-dependent control of SREBP2 cleavage is impaired, which results in deregulation of metabolic feedback of this pathway. This concept is consistent with the accumulation of cholesterol observed in clinical PCas samples. As a possible explanation for deregulated cholesterol synthesis in PCa, AKT1 and downstream mTORC1 signaling have been shown to have a prominent role in regulating the activation of the SREBP-controlled cholesterologenic program. Taken together, these reports point toward a mechanism by which PCa cells are able to maintain cholesterol de novo synthesis and uptake fully active or enhanced proliferation.

In the setting of enhanced SREBP2 activation and cholesterol production, LXRs could represent a defense mechanism to limit cellular cholesterol accumulation and to suppress tumor progression. Under these conditions, LXRs are activated by a specific panel of “LXR-agonists” oxysterols and/or some intermediates of the cholesterol biosynthetic pathway, such as desmosterol. This promotes cholesterol efflux and limits further accretion of lipoprotein-derived cholesterol. Interestingly, a similar idea was recently put forward to explain how LXRβ may limit dissemination of melanoma. In our study, we showed that LXR endogenous ligand accumulation in response to *PTEN* loss results in suppression of invasion and metastatic spreading of PCa. Whether this is dependent on a specific LXR-regulated gene or the result of the combinatorial effect of LXR activation remains unknown so far. It is interesting to note that in melanoma, induction of *ApoE* expression both in tumor and stromal cells seems to explain, at least in part, the effect of LXR activation. Beyond its role in transporting cholesterol, ApoE can also bind LRPs and LRPs on tumor and endothelial cells, respectively, and thus constrain cancer cell invasion and endothelial recruitment. Whether this underlies the effect of LXRs in PCa remains to be studied. However, other LXR target genes may also underlie the suppressive activity of LXRs during prostate carcinogenesis. For example, induction of the cholesterol efflux transporter ABCG1 by LXR agonists has been shown to attenuate signaling pathways emanating from lipid rafts, and specifically the PI3K pathway in LNCaP cells. Thus, loss of LXRs in vivo may result in enhanced pro-oncogenic signaling from lipid rafts to facilitate cancer progression. Clearly, identification of the bona fide LXR target gene(s) able to constrain *PTEN*-driven carcinogenesis is an important issue. This could allow better understanding of the link between cholesterol homeostasis and carcinogenic processes, and could provide new target(s) for alternative therapeutic strategies to treat advanced PCa.
Despite compelling evidence for their antitumor activity\textsuperscript{10, 12–14}, the roles of LXRs in tumors in general, and more specifically in PCa, are not well defined. Although we clearly demonstrate that LXRs control intrinsic invasiveness properties of epithelial prostate tumor cells using implanted grafts in athymic mice, one cannot rule out an important role for LXRs in the tumor microenvironment. In this setting, Villablanca et al.\textsuperscript{20} have reported that production of LXR ligands by various tumor cells impairs dendritic cell recruitment through inhibition of CCR7, which inhibits immune cells antitumor response. This observation is supported by a study reporting that 27-OHC, an established LXR ligand, activates LXR and promotes metastatic

**Fig. 6** LXRs control invasiveness of tumor cells through upregulation of EMT and metalloproteases. a, b Relative expression and protein accumulation of EMT markers in prostate from wild type (WT), Pten\textsuperscript{pc−/−} and Pten\textsuperscript{pc−/−} lxr\textsuperscript{αβ−/−} mice (N = 10/7/8). c Immunofluorescence staining against EMT markers VIMENTIN and SNAI1, together with Ki67. Nuclei are stained using Hoescht (blue). d Relative expression analysis of metalloproteinase genes Mmp1, 2, 7, 9 and negative regulator Timp2 in prostate from wild type (WT), Pten\textsuperscript{pc−/−} and Pten\textsuperscript{pc−/−} lxr\textsuperscript{αβ−/−} mice (N = 10 per group). e MMP9 protein accumulation in prostate from wild type (WT), Pten\textsuperscript{pc−/−} and Pten\textsuperscript{pc−/−} lxr\textsuperscript{αβ−/−} mice. f Histological and immunofluorescence staining for MMP9 in lung from Pten\textsuperscript{pc−/−} and Pten\textsuperscript{pc−/−} lxr\textsuperscript{αβ−/−} mice. White arrows indicate the positive MMP9 staining surrounded metastatic cells in contact with matrix. Asterisks identify metastatic cells and black arrows the surrounded pneumocytes. Nuclei are stained using Hoescht (blue). All data are represented as mean ± SEM and statistical analyses were performed with the Student’s t-test; **p < 0.01; ***p < 0.001. See also Supplementary Fig. 13.
LXRs downregulate intrinsic invasiveness properties of PTEN-negative tumor cells. a) Boyden chamber assays performed using PC3 cells transduced with SULT2B1b or Mock construct. Migrated cells are stained with DAPI in unstained (N=8), scale bar 100 µm. b) Description for renal subcapsular implantation in immunocompromized host mice (Nude mice) of prostate specimen from Ptenpc−/− and Ptenpc−/−/Lxrαβ−/− mice. c, d) Representative histological features (HE) and Ki67 staining of implant after 1 month of growth, and quantification of Ki67-positive cells from Ptenpc−/− (N=6) and Ptenpc−/−/Lxrαβ−/− (N=6) grafts on Nude mice, scale bar 100 µm. e) Evaluation of metastatic dissemination on lumbar lymph nodes and lung using prostatic markers CK18 and PSCA in grafted nude mice (N = 6 per group), nuclei are stained using Hoescht (blue), scale bar 100 µm. f) Recapitulative table of metastatic phenotype in lungs and lumbar lymph nodes of grafted Nude mice. All data are represented as mean ± SEM and statistical analyses were performed with the Student’s t-test; **p < 0.001.

Dissemination in breast cancer is not observed in vivo in colon cancer, clearly demonstrating that the role of LXRs in cancer is likely to be tumor-type specific. Overall, the effect pro- and anti-tumor effect of cholesterol metabolism should be carefully addressed given there are dependent on various parameters such as cell compartment, cancer type and enzymatic environment.

Interestingly, dendrogenin A, an enzymatic product of 5,6-epoxycholesterol, conjugated with histamine exhibits re-differentiation and growth control properties that improved animal survival. A parallel between the present report and dendrogenin A effects highlights that a better understanding of metabolic branches of cholesterol together with LXR signaling activity is a key point in cancer biology. In PTEN-null adenoscarcinomas, the origin of 5,6-epoxycholesterol is still unclear. 5,6-Epoxycholesterol could be metabolized by different enzymes to other active metabolites that may modulate LXRs activities. Indeed, the 3β-sulfated form of 5,6-epoxycholesterol and also 7-ketocholesterol produces antagonist ligands of the LXRs. Moreover, 3β-sulfated 5,6-epoxycholesterol is a transcriptional modulator of the LXRβ in breast cancer cells and mediates the LXRβ-dependent cytotoxic activity of molecules used in the clinic for breast cancer treatment such as Tamoxifen. Finally, LXR agonist production in PTEN-null cancer cells results partly from lipoperoxidation as emphasized by vitamin E supplementation experiments. These findings indicate that the redox status of cancer cells in absence of PTEN needs to be considered with respect to LXR signaling activity.

Our results also show that LXR activation is associated with the control of EMT in Pten-mutant tumor cells. These observations are in agreement with accumulation of SNAIL in Lxrαβ−/− mouse prostate as reported by Kim et al. in benign prostate hyperplasia. In combination with a PTEN mutation, in vivo genetic ablation of LXRs increases EMT and thus facilitates progression of prostatic carcinoma towards metastasis. Nevertheless, how LXRs control gene expression involved in the EMT process remains unclear. One possibility, alluded to above, is that this could be the result of altered lipid-raft-derived signaling in the absence of LXRs. Supporting this hypothesis, cholesterol medium enrichment increases accumulation of transforming growth factor-β (TGFβ) receptors in rafts and enhances their downstream signaling. Conversely, cholesterol lowering/depleting agents may change the binding ratio of TGFβ to TGFβ-R1 and -R2, which critically balances activity of this pathway. We thus speculate that absence of LXRs may increase cholesterol accumulation in lipid rafts, and that this in turn can stimulate TGFβ and downstream Smad signaling resulting in enhanced EMT, as we observe in Ptenpc−/−/Lxrαβ−/− prostate.

Fig. 7 LXRs downregulate intrinsic invasiveness properties of PTEN-negative tumor cells. a) Boyden chamber assays performed using PC3 cells transduced with SULT2B1b or Mock construct. Migrated cells are stained with DAPI in unstained (N=8), scale bar 100 µm. b) Description for renal subcapsular implantation in immunocompromized host mice (Nude mice) of prostate specimen from Ptenpc−/− and Ptenpc−/−/Lxrαβ−/− mice. c, d) Representative histological features (HE) and Ki67 staining of implant after 1 month of growth, and quantification of Ki67-positive cells from Ptenpc−/− (N=6) and Ptenpc−/−/Lxrαβ−/− (N=6) grafts on Nude mice, scale bar 100 µm. e) Evaluation of metastatic dissemination on lumbar lymph nodes and lung using prostatic markers CK18 and PSCA in grafted nude mice (N = 6 per group), nuclei are stained using Hoescht (blue), scale bar 100 µm. f) Recapitulative table of metastatic phenotype in lungs and lumbar lymph nodes of grafted Nude mice. All data are represented as mean ± SEM and statistical analyses were performed with the Student’s t-test; **p < 0.001.
Collectively, this study highlights LXRs as potent tumor suppressors in PCa and as a key determinant of prostate carcinogenesis and metastatic spread. Their effect is maintained by a marked metabolic shift in Pten-null PCa cells involving enhanced cholesterol biosynthesis. These findings support the development of therapeutic strategies that target cholesterol metabolism, next to currently established treatment modalities that target androgen receptor signaling. Given their ability to limit metastatic spread, LXRs are potential therapeutic targets in metastatic PCa, and that we need to reconsider the use of LXR agonists despite their side effects such as hypertriglyceridemia. Combining hormonal treatment agents with LXR ligands could represent a promising approach to treat metastatic PCa, which warrants further study.

**Methods**

**Animals experiments.** All mouse studies were conducted according to standard handle and care in agreement with the local ethic committee C2E2A. For simplicity, PB-cre<sup>−/−</sup>/Pten<sup>fl/fl</sup> mice were referred as WT and PB-cre<sup>+</sup>/Pten<sup>fl/fl</sup> as Pten<sup>−/−</sup>, Pten<sup>−/−</sup> mice were provided by Dr. David Mangoldorff's Lab (University of Texas Southwestern, Dallas, TX), Pten<sup>−/−</sup>/Lxrα<sup>−/−</sup> and Pten<sup>−/−</sup>/Lxrβ<sup>−/−</sup> were obtained by breeding the two lastest transgenic strains. Pten<sup>−/−</sup>/Lxrα<sup>−/−</sup>Lxrβ<sup>−/−</sup> control littersmates as referred as Lxr<sup>−/−</sup> in the manuscript. For simivastatin treatment experiments, mice were gavaged methylcellulose or simivastatin (Sigma-Aldrich) at 40 mg kg<sup>−1</sup> three times a week 1 month. For subrenal grafting experiments, 2-month-old mice (Charles River) were used. Dorsal prostates were implanted, and Pten<sup>−/−</sup> and Pten<sup>−/−</sup>/Lxrα<sup>−/−</sup>Lxrβ<sup>−/−</sup> aged of 6 months were collected and then freshly grafted under the renal capsule of anesthetized male nude mice. After prostate implantation and kidney repositioning, mice were sutured on skin and muscle planes. Prostatic grafts were collected 1 month later. Lung and lumbar lymph nodes were analyzed for metastasis development by hematoxylin/eosin coloration and immunohistochemistry.

**Reagents.** T0901317 ligand was purchased from Cayman Chemical (71810). Simvastatin (56196), Doxycycline (D9891) and Vitamine E (T3376) were purchased from Sigma-Aldrich, Wortmannin (#9951) and LY294002 (#9901) from Dr. Marc Poirot group51, 52. In brief, for the oxysterols analysis was samples were converted to trimethylsilyl ethers by treatment with 130 µl Sylon HTP (hexamethyldisilylazane:trimethyl-chlorosilane:pyridine, 3:1:9) (Supelco, Bellefonte, PA) at 37 °C for 45 min. Solution was homogenized using a syringe with 19 G needle and plate in DMEM (Sigma-Aldrich) supplemented with FBS 10%. Oxysterols were determined by Gas chromatography–mass spectrometry (GC–MS) using deuterium-labeled internal standards provided by Dr. Marc Poirot group51, 52. In brief, for the oxysterols analysis was samples were converted to trimethylsilyl ethers by treatment with 130 µl Sylon HTP (hexamethyldisilylazane:trimethyl-chlorosilane:pyridine, 3:1:9) (Supelco, Bellefonte, PA) at 37 °C for 45 min. Solution was homogenized using a syringe with 19 G needle and plate in DMEM (Sigma-Aldrich) supplemented with FBS 10%. Oxysterols were determined by Gas chromatography–mass spectrometry (GC–MS) using deuterium-labeled internal standards provided by Dr. Marc Poirot group51, 52. In brief, for the oxysterols analysis was samples were converted to trimethylsilyl ethers by treatment with 130 µl Sylon HTP (hexamethyldisilylazane:trimethyl-chlorosilane, 3:1:9) (Supelco, Bellefonte, PA) at 60 °C for 30 min. After incubation, the solution was evaporated under a stream of nitrogen and the residue dissolved in n-hexane and transferred to an autosampler vial. Analyses were performed on an Agilent 6890N GC equipped with a HP-5 capillary column (0.25 mm, 0.25 mm, 0.25 mm) and a flame ionization detector (Agilent Technologies, Palo Alto, CA). Separation was carried out on a 30 m capillary column (HP-5MS 30 m x 0.25 mm ID, 0.25 mm thickness). Quantification of oxysterols was made by the isotope dilution method.

**Human data set analysis.** Box plots and heatmap were performed using ONCOMINE portal (www.oncomine.org). GSEA has been used with a list of 24 genes (e.g., genes with functionally characterized LXRE in human promoter sequence). Analysis was performed using http://www.broadinstitute.org platform with GSEA software v2.2.34, 35. When more than one probe are available in the dataset, the probe with maximum signal was used.

**Mouse embryonic fibroblasts.** MEFs have been derived from 13.5 dpc embryos WT, Lxrα<sup>−/−</sup>, Lxrβ<sup>−/−</sup> or Lxrαβ<sup>−/−</sup>. Briefly, dorsal part of the dissected embryos have been sliced and incubated with PBS Trypsin-EDTA digestion mix (Sigma-Aldrich) at 37 °C for 45 min. Solution was homogenized using a syringe with 19 G needle and plate in DMEM (Sigma-Aldrich) supplemented with FBS 10% (Biowest), glutamine 2 mM (Sigma-Aldrich), minimal essential medium non-essential amino acids solution (Sigma-Aldrich), Streptomycin 100 µg ml<sup>−1</sup> (Sigma-Aldrich) and Penicillin 100 µg ml<sup>−1</sup> (Sigma-Aldrich). MEFs have been plated in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. MEFs have been split upon confluence using 0.05% trypsin/EDTA solution (Sigma-Aldrich). Cells were counted using ImageJ software.

**Cholesterol and precursors measurement.** To a screw-capped vial sealed with a Teflon septum, sample homogenates were added together with 250 ng

**SULT2B1b cells were seeded in the upper chamber in minimum medium RPMI-1640 medium (Invitrogen) containing penicillin/streptomycin (100 µg ml<sup>−1</sup>), L-glutamine (2 mM). The lower chamber contain RPMI-1640 medium (Invitrogen) supplemented with penicillin/streptomycin (100 µg ml<sup>−1</sup>), L-glutamine (2 mM) and 10% FBS (Biowest). Cells were incubated for 48 h before fixation using Paraformaldehyde 4% and staining with Hematoxylin (Sigma). Remaining cells in upper chamber were removed with cotton tips. Cell number has been counted using ImageJ software.

**Western blot analysis.** Proteins were extracted from tissues and cells using a buffer solution with Heps 25 mM, NaCl 400 mM, MgCl<sub>2</sub> 1.5 mM, EDTA 200 mM, Nonidet-P40 1% supplemented with phenylmethylsulfonyl fluoride 5 µM, Na<sub>3</sub>VO<sub>4</sub> 0.1 NaF, 0.1 mM and complete protease inhibitor cocktail (Roche Diagnostics, Meylan, France). Lysates were resolved on SDS-polyacrylamide gels. Blotting and transferred to nitrocellulose membrane (Hybond–ECL, GE Healthcare). Membranes were incubated overnight at 4°C with primary antibodies. Detection was performed using a peroxidase-conjugated anti-rabbit or mouse immunoglobulin G (P.A.R.L.S) and the Western Lightning System (Roche Diagnostics). Uncropped pictures of western blottings are available in Supplementary Fig. 14. Antibodies used for western blots are listed in Supplementary Table 1.
D7-lathosterol, 500 ng of D6-desmosterol, 100 ng of D6-lanosterol, 20 ng D7-7a-hydroxysterol, D7-7β-hydroxysterol, D7-7 ketosterol, D3-24α-hydroxysterol and D6-27-hydroxysterol, and 10 μg of D6 cholesterol internal standards, 50 μl butylated hydroxytoluene (5 g l⁻¹) and 50 μl EDTA (10 g l⁻¹) to each vial and flushed with argon for 20 min to remove air. Alkaline hydrolysis was allowed to proceed at room temperature (22 °C) with magnetic stirring for 1 h in the presence of ethanolic 1 M potassium hydroxide solution. After hydrolysis, the sterols were extracted twice with 5 ml cyclohexane. The organic solvents were evaporated under a gentle stream of argon, and sterols and cholesterol separated by solid phase extraction with 3 ml of hexane + 0.5% isopropanol and oxysterols with 5 ml of hexane + 30% isopropanol. Sterols and oxysterols were converted into trimethylsilyl ethers with N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) with trimethylchlorosilane 1% (Pierce). GC–MS analysis was performed on GC equipped with an Elite column (30 m × 0.32 mm id × 0.25 mm film; Perkin Elmer, USA) and injection was performed in splitless mode and using helium (1 ml min⁻¹) as a carrier gas. The temperature program was as follows: initial temperature of 180 °C was held for 1 min, followed by a linear ramp of 20 °C min⁻¹ to 270 °C, and then a linear ramp of 5 °C min⁻¹ to 290 °C, which was held for 10 min. The mass spectrometer operates in the selected ion-monitoring mode. Peak integration is performed manually and sterols are quantified from selected-ion monitoring analyses against internal standards using standard curves for the listed sterols. Additional qualifier (characteristic fragment) ions were used for structural identification.

Supernatant steroid contains assay. PCs cells transduced with empty vector, pGFP–PTEN, pGFP–PTENC124A or pCMV–HA–AKT and/or vector P, pCMV–Luc reporter construct and expression vector pCMX–GaHLR6–LRD or pCMV–GaHLR6–LRD. FBS 10% and T901317 (1 μM) treatments have been performed as a control.

Microarray analyzes. Biotinylated single strand complementary DNA targets were prepared, starting from 250 ng of total RNA, using the Ambion WT Expression Kit (catalog number 4411974) and the Affymetrix GeneChip WT Terminal Labeling Kit (catalog number 900671) according to Affymetrix recommendations. Following fragmentation and end labeling, 3 μg of cDNAs were hybridized for 16 h at 45 °C by reduced apoptosis. Cell Metab. 19, 393–406 (2016). De Bousac, H. et al. Oxysterol receptors and their therapeutic applications in cancer conditions. Expert. Opin. Ther. Targets 17, 1029–1038 (2013). Dufour, J. et al. Nuclear receptor X receptors leads to cell proliferation in a model of metastatic prostate cancer xenografts. PLoS ONE 8, e58767 (2013). Chua, C. et al. Inhibition of tumor growth and progression of LNCaP prostate cancer cell line by androgen and liver X receptor agonist. Cancer Res. 66, 60–66 (2006). Pommier, A. J. C. et al. Liver X receptor activation downregulates AKT survival signaling in lipid rafts and induces apoptosis of prostate cancer cells. Oncogene 36, 2172–2185 (2010).

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Author contributions

S.B. and S.B. supervised the experiments. S.B., A.S. and P.V. performed statistical analysis. A.A., A.F., A.I. and M.M. performed investigations on cholesterol homeostasis in vitro and in vivo. S.B., A.A. and J.D. performed statistical analysis. S.B., A.A. and J.D. performed the experiments. S.B. supervised experiments. S.B., A.S. and P.V. performed computational analysis. A.A., A.F., A.I. and M.M. performed investigations on cholesterol homeostasis in vivo. S.B., A.A. and J.D. performed the experiments.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

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