Akt-Mediated Phosphorylation and Activation of Estrogen Receptor α Is Required for Endometrial Neoplastic Transformation in *Pten*$^{+/-}$ Mice

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Abstract

***PTEN* is a tumor suppressor gene frequently mutated in human cancers. *In vitro* and *in vivo* studies have shown that *PTEN* can exert its tumor suppressive function through a variety of mechanisms, including regulation of cell death and cell proliferation. However, it is still unclear which of the many downstream pathways are critical in each different tissue, *in vivo*. Loss of *PTEN* is the earliest detectable genetic lesion in the estrogen-related type I (endometrioid) endometrial cancer. *Pten*$^{+/-}$ mice develop endometrial neoplastic lesions with full penetrance, thus providing a model system to dissect the genetic and biochemical events leading to the transition from normal to hyperplastic and neoplastic endometrial epithelium. Here, we show that loss of *Pten* in the mouse endometrium activates Akt and results in increased phosphorylation of estrogen receptor α (ERα) on Ser^{167}. ERα phosphorylation results, in turn, in the activation of this nuclear receptor both *in vivo* and *in vitro*, even in the absence of ligand, and in its increased ability to activate the transcription of several of its target genes. Strikingly, reduction of endometrial ERα levels and activity dramatically reduces the neoplastic effect of *Pten* loss in the endometrium, in contrast to complete estrogen depletion. Thus, we provide for the first time *in vivo* evidence supporting the hypothesis that loss of *Pten* and subsequent Akt activation result in the activation of ERα-dependent pathways that play a pivotal role in the neoplastic process.** (Cancer Res 2006; 66(7): 3375-80)

Introduction

The *PTEN* tumor suppressor gene encodes a protein/lipid phosphatase whose main *in vivo* function is to convert phosphatidylinositol (3,4,5)-triphosphate (PIP-3), the product of phosphatidylinositol 3-kinase (PI3K) activity, into phosphatidylinositol (4,5)-biphosphate (1). The levels of PIP-3 rapidly increase upon growth factor stimulation and activation of PI3K, allowing recruitment and activation of proteins containing a pleckstrin homology domain. Among these is the Akt kinase that, upon membrane recruitment, is activated by phosphorylation (2). AKT, in turn, phosphorylates an ever-growing list of target proteins regulating key processes, such as proliferation, survival, cell size, and mRNA translation (2). Somatic deletions or mutations of *PTEN* have been identified in a large fraction (12-60%) of human tumors (3). Loss of *PTEN* is mostly associated with advanced-stage tumors. However, the endometrioid subtype of endometrial cancer (EEC) represents one remarkable exception. EEC is estrogen related, low grade, develops in premenopausal and perimenopausal women, and is often preceded by complex atypical endometrial hyperplasia (4). *PTEN* is completely lost or mutated in >50% of primary EECs (5) and in at least 20% of endometrial hyperplasias, the precancerous lesions of the endometrium (5, 6). Thus, loss of *PTEN* is a very early event in the multistep process leading to EEC.

Consistent with the human tumor data, *Pten*$^{+/-}$ mouse mutants, in the mixed C57/BL6x129/Sv genetic background, show a striking susceptibility to develop a broad array of tumors (7, 8), including endometrial complex atypical hyperplasia and carcinoma (9, 10). The endometrial lesions are dramatically accelerated by the simultaneous inactivation of *p27KIP1* (11) or *Mlh1* (12), further supporting the current multistep models of endometrial tumorigenesis.

The goal of this study was to further exploit the *Pten*$^{+/-}$ mouse model to investigate the mechanisms of endometrial neoplastic transformation upon loss of Pten.

Materials and Methods

Animals and treatments. Generation of *Pten*$^{+/-}$ mice has been described (7). Ovariectomy was done on 3-week-old C57/BL6x129/Sv mice and on 14-week-old 129/Sv mice. ICI 182,780 (AstraZeneca, Wilmington, DE) was injected s.c. weekly at 0.5 mg/mouse. For diethylstilbestrol treatment, newborn mice were injected s.c. with 2 μg of diethylstilbestrol in corn oil for five consecutive days as described (13).

Luciferase assays. Ishikawa EEC cells and their derivative IT-3015 (14) were grown in DMEM/10% fetal bovine serum (FBS). Transfections were done with GeneJammer (Stratagene, La Jolla, CA) according to the manufacturer's protocol. Cells were grown for 24 hours in E_2-free medium and transfected in sextuplicate with 800 ng of ERE2-TK-LUC reporter (containing a luciferase gene regulated by two estrogen response elements) with or without 200 ng of pCMV5-ERα, pCMV5-ERα^{S167A}, pcDNA-PTEN, pcDNA-PTEN^{C124S}, pCMV5-MyrAKT. Three hours after transfection, the cells were treated with or without E_2 (100 nmol/L) and/or doxycycline (2 μg/mL) for 36 hours. Luciferase activity was measured with the Promega Luciferase kit (Madison, WI) according to the manufacturer's recommendations. Values were normalized by protein content because Akt activation strongly affects both the expression of *Renilla* luciferase–based constructs and the pCMV-βgal reporter (data not shown).

Immunohistochemical analysis. The rabbit polyclonal antibodies used were anti-Pten (NeoMarkers, Fremont, CA), anti-phospho-AKT S473, and anti-phospho-ERα S167 (Cell Signaling, Beverly, MA). Tissues were fixed in buffered 10% formalin, embedded in paraffin, and sectioned at 6 μm. Sections were subjected to antigen retrieval in 0.1 mol/L sodium citrate and counterstained with hematoxylin.

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Primary endometrial epithelial cells isolation. Uteri from age-matched wild-type and *Pten*$^{+/-}$ cohorts were removed, pooled, and immediately minced in 1-mm fragments. The tissues were incubated in 50-mL conical tubes containing 0.5% collagenase and 0.1% DNase II in HBSS for 30 to 45 minutes at 37°C, with shaking. The tubes were then kept vertical for 10 minutes at room temperature to settle the glands. The top two thirds of the supernatant, containing stromal cells, was removed, and DMEM/10% FBS was added to the glands. This procedure was repeated thrice, resulting in a final pellet of highly purified endometrial glands.

Real-time PCR. Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA) from pooled primary endometrial epithelial cells. Two micrograms of RNA were reverse transcribed with a commercially available kit (Thermoscript, Invitrogen). cDNAs were diluted 10-fold, and 10 μL were used for real-time PCR on the ABI Prism 7900 Sequence Detection System using Taqman Master mix and primers (Applied Biosystems, Foster City, CA). Each sample was run in triplicate, and β-actin was used to control for input RNA. Fluorescence was measured with the Sequence Detection Systems 2.0 software. After adjusting with the β-actin control, the difference in cycle crossing points was calculated for each gene between the wild-type group and mutants. For a theoretical efficiency of 100%, the fold difference was calculated by 2 to the power of the cycle point difference. Experiments were repeated at least twice.

Results and Discussion

To circumvent the phenotypic variability associated with mixed genetic backgrounds, we have bred the *Pten* allele into a pure 129/Sv strain. All *Pten*$^{+/-}$ female mice in this genetic background develop, starting at 3 months of age, multiple endometrial hyperplastic lesions characterized by stratification of the glands; dramatically enlarged epithelial cells showing eosinophilic cytoplasm, nuclear atypia, and abnormal cell polarity (Fig. 1*D*); dilation of the gland lumen; papillary formation; and increasing gland/stromal ratio. Complete loss of Pten expression is very common even in small early lesions (data not shown). Between 5 and 9 months of age, these lesions progress from extensive atypical hyperplasia to *in situ* carcinomas. Locally invasive carcinomas, defined by clear myometrial/serosal invasion, occur in ~30% of the animals. Most animals succumb by the 10th month of age due to extensive hemorrhage and necrosis consequent to the multifocal obliteration of the uterine horns. These characteristics closely mimic both the natural history and the pathologic features of human EEC (Fig. 1*G*), of which this mouse is a valuable model.

Human EEC usually develops in a context of unopposed estrogen activity (high circulating levels of estrogen with no or low levels of progesterone), such as unopposed estrogen replacement therapy, obesity, polycystic ovarian syndrome, nulliparity, and late menopause. Conversely, *Pten*$^{+/-}$ mice are fertile and have a normal estrous cycle and normal hormone serum levels (data not shown).

The development of estrogen-related neoplastic lesions in the absence of detectable hyperestrogenism suggests that there might exist a functional and physiologic link between loss of Pten and increased estrogen receptor (ER) activity. Several *in vitro* studies have suggested the existence of a crosstalk between Akt and ERα (15, 16). Akt can phosphorylate and activate ERα at Ser^{167}, in the ligand-independent transcriptional activation domain (AF1), at least *in vitro* and in cell culture systems (17, 18). Nevertheless, *in vivo* data defining the existence and physiologic relevance of this interaction, as well as its role in EEC pathogenesis, are still missing.

To assess whether this functional link exists *in vivo*, we did immunohistochemistry on serial uterine sections from wild-type and *Pten* heterozygous mice using antibodies recognizing the phosphorylated, active forms of both Akt (Ser^{473}) and ERα (Ser^{167}). The endometrial glands of wild-type mouse uteri express low levels of both active Akt- and Ser^{167}-phosphorylated ERα (Fig. 1*B* and *C*). However, the hyperplastic endometrial glands from age-matched *Pten* heterozygous females showed strikingly increased phosphoprotein levels of both Akt and ERα, suggesting a direct causal relationship between Akt activation and ERα phosphorylation *in vivo* (Fig. 1*E* and *F*). This correlation was also maintained in a

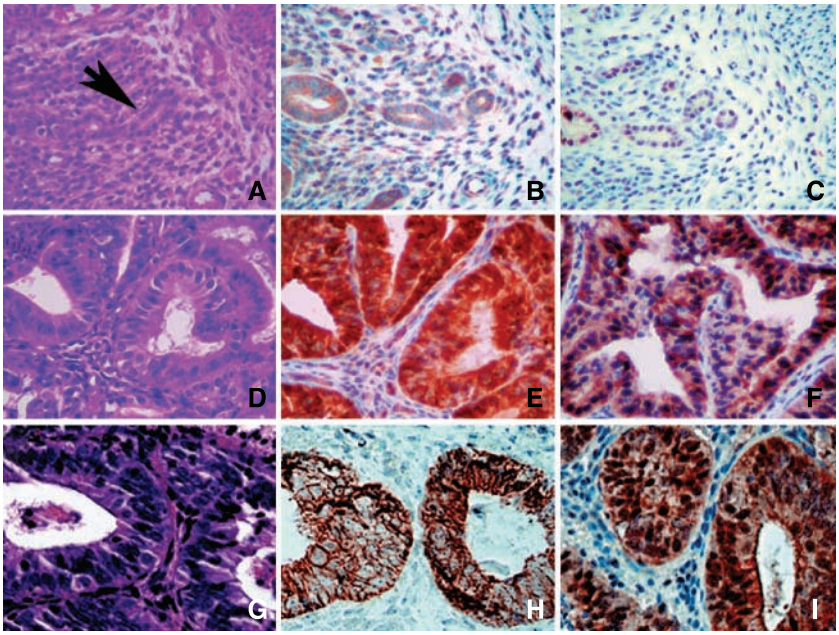

Figure 1. Loss of *Pten* in the mouse and human endometrial epithelium is associated with Akt (S473) and ERα (S167) phosphorylation. Representative sections of murine wild-type (*A-C*) and *Pten*$^{+/-}$ (*D-F*) endometrium at 25 weeks of age compared with a human grade 1 tumor (*G-I*), stained with H&E (*A, D*, and *G*) to show the glandular epithelium (*A, arrow*), with anti-phospho-Ser^{473}-Akt (*B, E*, and *H*) and anti-phospho-Ser^{167}-ERα (*C, F*, and *I*). Note the dramatic increase in cell size upon loss of Pten (*A* and *D*). Original magnification, ×400.

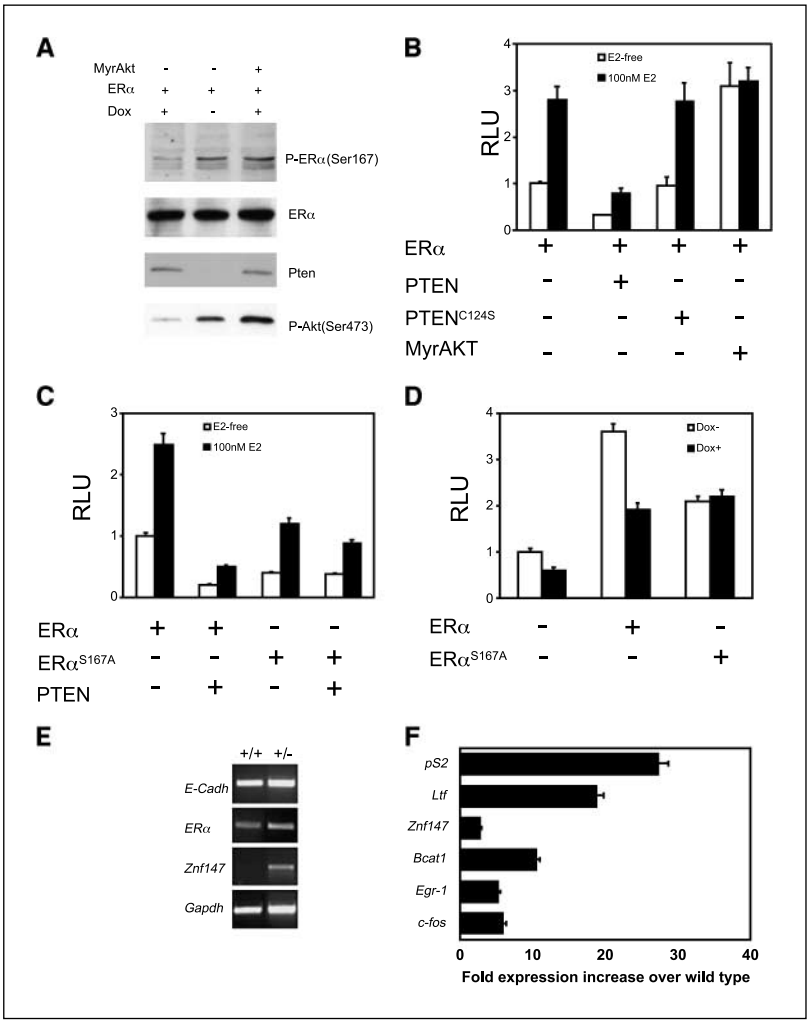

Figure 2. Activated Akt phosphorylates ERα, resulting in increased transcriptional activity, even in the absence of estrogen. *A,* Western blot analysis of ERα phosphorylation in IT3015 cells in the presence or absence of activated Akt. *B-D,* luciferase assays done in Ishikawa (*B* and *C*) and IT3015 cells (*D*) transiently transfected with an estrogen-dependent reporter and the indicated expression vectors. *E,* RT-PCR determination of the expression levels of *E-cadherin, ERα, Efp/Znf147*, and *Gapdh* in isolated primary endometrial epithelial cells. *F,* real-time PCR analysis of the increase in expression of a series of estrogen target genes in primary endometrial epithelial cells, normalized to β-actin expression.

small pilot group of nine human grade 1 EEC samples selected based on loss of PTEN expression. As expected, all cases analyzed expressed high levels of phospho-AKT (Fig. 1*H*). Seventy-five percent of cases had strong phospho-ERα immunoreactivity (Fig. 1*I*), suggesting that loss of PTEN, activation of AKT, and phosphorylation of ERα may be related events also in human EEC.

To formally prove that activated Akt is able to phosphorylate ERα in endometrial epithelial cells, we did transient transfection experiments in IT3015, a subclone of the *PTEN* null Ishikawa EC cell line, engineered to reexpress *PTEN* upon doxycycline treatment. Both the IT3015 cells and the original Ishikawa strain in our possession express very low levels of endogenous ERα. When we transfected IT3015 cells with wild-type human ERα, we could readily detect receptor phosphorylation at Ser^{167} by Western blot, using a phospho-specific antibody (Fig. 2*A*). However, drastic reduction of AKT phosphorylation upon *PTEN* induction resulted in a parallel decrease in the phosphorylation of ERα. This inhibition was bypassed by the expression of a myristoylated, constitutively active form of AKT, which is not affected by the presence of PTEN. These data further confirm that loss of *PTEN* in EC cells results in the phosphorylation of ERα through the activation of AKT.

We next asked whether Ser^{167} phosphorylation would affect ERα-mediated transcription in EC cells. First, we transiently transfected Ishikawa cells with an estrogen responsive reporter (ERE2-TK-LUC) together with a plasmid expressing wild-type *PTEN* or a catalytically inert mutant, C124S. As expected, the transcriptional response was increased in cells treated with estradiol, compared with cells grown in medium depleted of steroid hormones (Fig. 2*B*). Expression of *PTEN*, but not of the C124S mutant, reduced the basal (hormone-free) ERα transcriptional activity, whereas expression of the constitutively active AKT mutant increased the basal ERα activity to the same levels observed in the presence of hormone. Conversely, an ERα mutant that cannot be phosphorylated by AKT ($ERα^{S167A}$) showed an overall reduced transcriptional activity, which was not further reduced by cotransfection of *PTEN* (Fig. 2*C*). Finally, a related experiment done in the IT3015 inducible cell line confirmed that down-regulation of AKT activity, consequential to *PTEN* reexpression, reduced wild-type ERα transcriptional activity to levels comparable with those obtained using $ERα^{S167A}$, and that $ERα^{S167A}$-mediated transcription was not altered by *PTEN* expression (Fig. 2*D*). Collectively, these data show that loss of *PTEN* and AKT activation in EC cells result in a phosphorylation-dependent enhancement of ERα transcriptional activity, independent of the presence of estradiol.

We then asked whether this increase in ERα transcriptional activity upon Akt activation is also true *in vivo*, in a much more

Table 1. Estrogen depletion reduces but does not completely inhibit tumor formation

Genotype	Age (wk)	Treatment	No. mice	No. mice with lesions (%)	No. lesions per mouse (mean ± SD)	Size of lesion (mm^2)	Range of size (mm^2)	Ki-67 expression (%)
+/−	32	Ovariectomy	9	8 (90)	8.20 ± 10.27	0.13 ± 0.13	0.01→0.96	51.6 ± 36.84
+/−	32	Oil	9	9 (100)	18.56 ± 8.57	0.22 ± 0.12	0.04→0.75	78.12 ± 7.34
+/+	32	Ovariectomy	9	0	0	0	0	8.49 ± 3.98
+/+	32	Oil	4	0	0	0	0	69.95 ± 25.69

complex physiologic setting. First, we did semiquantitative reverse transcription-PCR (RT-PCR) on RNA extracted from purified endometrial epithelial cells. We tested the expression levels of E-cadherin, to confirm the epithelial nature of the purified cells and to further normalize for the amount of epithelial cells, and ERα, to exclude an effect of *Pten* loss on the expression of ERα *in vivo*. No differences were noticed between the expression levels of these genes in the two groups of mice (Fig. 2*E*). Next, we tested a well-established ERα transcriptional target, *Efp/Znf147*. Endometrial epithelial cells from $Pten^{+/-}$ mice expressed *Znf147* mRNA at much higher levels than wild-type cells, again strongly suggesting that loss of *Pten* results *in vivo* in increased ERα transcriptional activity (Fig. 2*E*). To better measure this effect, we employed real-time RT-PCR and compared the expression levels of a series of estrogen-induced genes in RNAs from wild-type and $Pten^{+/-}$ freshly isolated endometrial epithelial cells. Overexpression of *pS2, Egr-1, c-fos, Efp/Znf147, Bcat1*, and *Ltf* was readily detected in the mutant epithelium (Fig. 2*F*). Collectively, these data show that loss of *Pten* and activation of Akt result *in vivo* in a considerable increase in the mRNA levels of several estrogen target genes, suggesting that Akt-mediated phosphorylation of ERα can significantly enhance the receptor response to its ligand and thus contribute to the initiation of the neoplastic process.

As the effect of Akt-mediated phosphorylation on ERα transcriptional activity seems to be ligand independent, one important prediction from this model is that complete estrogen depletion will not be able to *entirely* inhibit the neoplastic process in the $Pten^{+/-}$ endometrial cells.

To determine to what extent estrogen contributes to the development of the endometrial neoplastic lesions in $Pten^{+/-}$ mice, we analyzed the uteri of 32-week-old $Pten^{+/-}$ mice (in the C57/BL6x129/Sv background, for this experiment only) that had been ovariectomized at 3 weeks of age. As predicted by our model, estrogen-depleted mice still developed endometrial tumors, although their average number and size was reduced by about 50%. Strikingly, estrogen depletion did not result in a significant change in the proliferation index in $Pten^{+/-}$ mice (Table 1). These results suggest that estrogen is not absolutely necessary for tumor development in cells expressing high levels of activated Akt and ERα but can anyway cooperate with Akt activation to facilitate the neoplastic process.

We next asked how relevant this crosstalk between the Pten/Akt pathway and ERα is for the tumorigenic process. To show *in vivo* that ERα is a key downstream effector of Akt, we attempted to down-regulate the receptor using a pure ERα antagonist, ICI 182,780. This compound produces a down-regulation of intracellular ER protein and an impairment of ER dimerization (19). We treated a group of 14-week-old 129/Sv $Pten^{+/-}$ mice (n = 10) with weekly s.c. injections of ICI 182,780 for 6 weeks. Untreated $Pten^{+/-}$ intact mice and untreated $Pten^{+/-}$ mice ovariectomized at the same age were used as controls. At 14 weeks, all untreated 129/Sv $Pten^{+/-}$ mice show few focal areas of hyperplasia (Fig. 3*A*), whereas at 20 weeks (our end point), untreated mice in this genetic background have developed multiple extensive lesions that fuse together to completely disrupt the uterine architecture (Fig. 3*A*).

The uteri of $Pten^{+/-}$ mice showed increased levels of phospho-Akt compared with intact wild-type mice and independent of ovariectomy or ICI treatment, thus proving that Akt activity was not affected by the treatments. The levels of ERα were slightly increased in ovariectomized mice, likely as a result of a release from an estrogen-induced negative feedback. Conversely, the protein levels of ERα were reduced ~4-fold in the ICI-treated cohort (Fig. 3*B*).

To evaluate the effect of ERα reduction/inhibition on tumor development, we scored the number of lesions in the uteri of ovariectomized and ICI-treated mice. At this stage (20 weeks of age), it was already impossible to score individual lesions in the uteri of untreated $Pten^{+/-}$ mice in the 129/Sv strain. Both ovariectomized and ICI-treated mice developed focal lesions (Fig. 3*A*), but the number of lesions in the ICI-treated females was less than half compared with ovariectomized mice (Fig. 3*C*). In addition, the proliferation index, determined by staining tissue sections with an antibody against the Ki-67 marker, was severely reduced by the ICI treatment but not by hormone depletion (Fig. 3*D*), as already discussed above. Real-time PCR on RNA extracted from the three cohorts showed that ICI treatment was more effective than estrogen depletion at reducing the expression of several markers of estrogenic activity (Fig. 3*E*). These results show that a decrease in ERα levels and activity can dramatically reduce the development of endometrial lesions in $Pten^{+/-}$ mice.

As a parallel approach, we took advantage of one long-term effect of the synthetic estrogen diethylstilbestrol in the mouse uterus. Diethylstilbestrol administration during postnatal days 1 to 5 elicits distinct alterations in several structures of the female reproductive tract, including scattered areas of metaplasia in the uterus and cervix and persistent cornification of the vaginal epithelium. Most importantly, adult mice that were exposed to diethylstilbestrol only in this neonatal time window show a significant reduction of ERα levels and an attenuation of the physiologic effects of estrogen action in the uterus, a phenotype that partially mimics ERα gene disruption as exhibited by the ERKO mice (20).

We injected diethylstilbestrol or control oil vehicle in newborn wild-type and $Pten^{+/-}$ mice in the 129/Sv genetic background (n = 8 per group) and sacrificed them at 25 to 27 weeks of age. By this time, the uterus of a 129/Sv $Pten^{+/-}$ mouse exhibits extensive endometrial lesions. The average uterine weight of the control

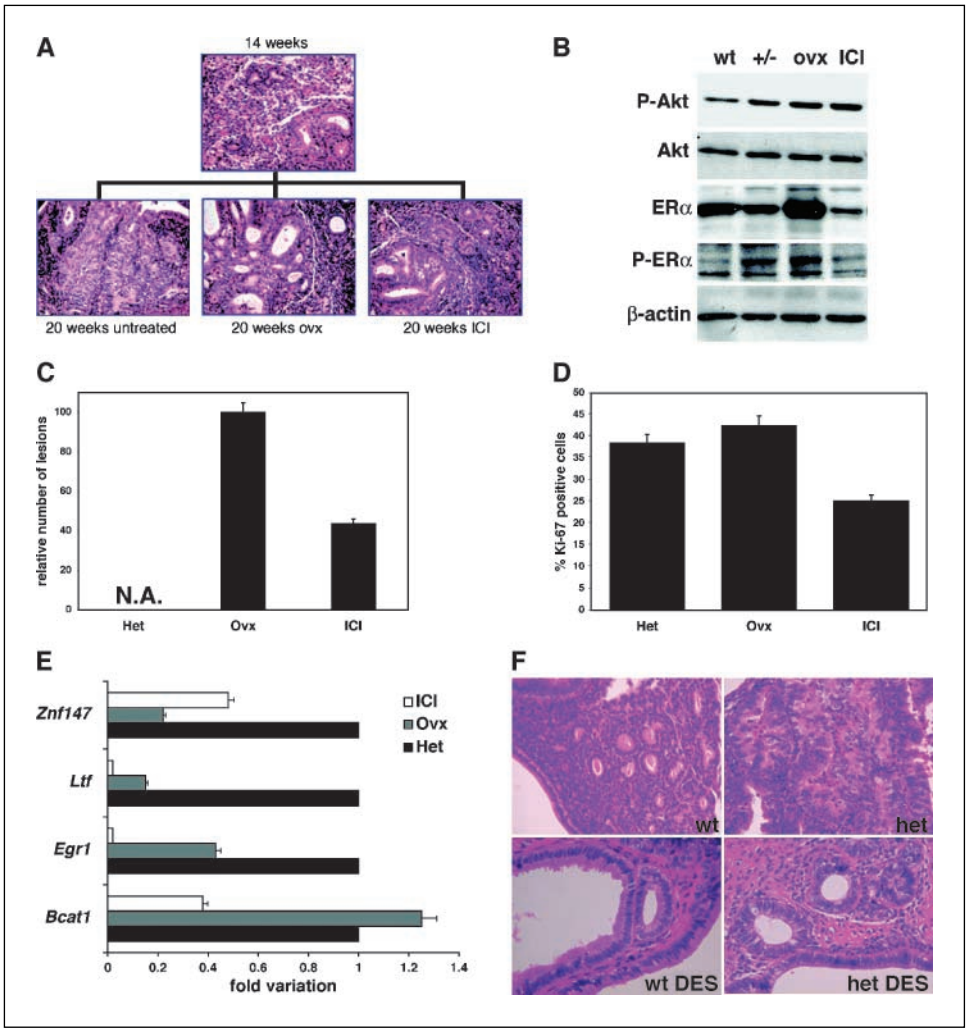

Figure 3. The pure ER antagonist ICI 182,780 can partially inhibit endometrial tumor development consequent to *Pten* loss. *A,* representative photomicrographs of the endometrium of *Pten*$^{+/-}$ mice at 14 weeks of age and at 20 weeks, after ovariectomy or ICI treatment. Original magnification, ×400. *B,* Western blot analysis of Akt phosphorylation and ERα levels and phosphorylation in the uterus of mice subjected to the indicated treatments. *C* and *D,* ICI treatment reduces the number of lesions and the proliferation index in the uteri of *Pten*$^{+/-}$ mice. *N.A.,* not available: lesion number could not be scored due to the extensive fusion of neoplastic areas and disruption of uterine architecture. *E,* real-time PCR analysis showing the decrease in expression of a series of estrogen target genes in the uterus of ICI-treated mice, normalized to β-actin expression. *F,* diethylstilbestrol (*DES*) treatment inhibits endometrial neoplastic transformation consequent to *Pten* loss: representative photomicrographs of wild-type and *Pten*$^{+/-}$ oil-treated controls and of wild-type and *Pten*$^{+/-}$ diethylstilbestrol-treated mice. Original magnification, ×400.

Pten$^{+/-}$ mice, normalized for their body weight, was 5-fold greater than that of the control wild-type mice (Table 2). However, reduction of the estrogenic response in sexually mature mutant mice, as a consequence of diethylstilbestrol treatment, resulted in the complete abolition of the hyperplastic features characterizing the uteri of age-matched *Pten*$^{+/-}$ mice. Real-time PCR showed that, as expected, ERα levels were severely reduced in the diethylstilbestrol-treated mice (Table 2). Strikingly, upon pathologic examination of tissue sections from the dissected uteri, we could not detect any neoplastic lesions in the diethylstilbestrol-treated *Pten*$^{+/-}$ cohort (Fig. 3*F*). Moreover, although several areas of complete loss of Pten expression were commonly found in the control mutant cohort, the uteri from the diethylstilbestrol-treated *Pten*$^{+/-}$ mice showed complete retention of Pten immunostaining, further confirming the absence of any neoplastic change (data not shown). Taken together with the ICI results, these data further

Table 2. Diethylstilbestrol treatment reduces ERα levels and completely inhibits uterine hyperplasia

	Control		DES*	
	Wild type	*Pten*$^{+/-}$	Wild type	*Pten*$^{+/-}$
Uterus weight/body weight (g)	0.378 ± 0.07 †	1.993 ± 1.04 †,‡	0.649 ± 0.16	0.692 ± 0.23 ‡
ERα relative expression	1	1.059	0.109	0.347

*Uterus weight in diethylstilbestrol-treated includes the weight of the cervico-vaginal tract, which is markedly thickened as a result of the treatment.
†$P < 0.001$.
‡$P < 0.003$.

support the hypothesis that inhibition/down-regulation of ERα can efficiently disrupt the oncogenic pathways initiated by Akt activation.

In summary, our data strongly suggest that supraphysiologic activation of ERα is, *in vivo*, an obligatory pathway for the development of endometrial lesions consequent to loss of *Pten* and activation of Akt. The functional connection between these molecules is further underlined by the fact that loss of PTEN is typically found in EECs expressing high levels of ERα (21). Similarly, the same axis is likely to play a pivotal role in the development of ERα-positive breast cancer. Although the association between loss of PTEN and ERα expression in this tumor type is less well established, with reports describing either a direct (22) or an inverse (23) correlation, it has to be noted that whereas loss of PTEN is relatively uncommon in early breast cancer, AKT activation is extremely frequent, as a consequence, for example, of PI3KCA activating mutations (24) or ErbB2 amplification (25), and is often associated to ERα-positive tumors (24, 26). Thus, in the case of ER-positive breast cancer, AKT-mediated activation of ERα may be independent of PTEN loss.

Although further effort is clearly warranted to unravel the relative contribution of the different ERα-dependent pathways to the neoplastic process, our results not only uncover a novel pivotal tumorigenic pathway but may also provide a rationale for the failure of treatments based on the use of aromatase inhibitors (27). In addition, our findings suggest that pure ERα antagonists, such as fulvestrant, alone or in combination with aromatase inhibitors, may be an effective treatment choice in EECs characterized by loss of PTEN and AKT activation.

Acknowledgments

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References

1. Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell 2000;100:387–90.
2. Bellacosa A, Kumar C, Di Cristofano A, Testa JR. Activation of AKT kinases in cancer: implications for therapeutic targeting. Adv Cancer Res 2005;94:29–86.
3. Ali IU, Schriml LM, Dean M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. J Natl Cancer Inst 1999;91:1922–32.
4. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983;15:10–7.
5. Sun H, Enomoto T, Fujita M, et al. Mutational analysis of the PTEN gene in endometrial carcinoma and hyperplasia. Am J Clin Pathol 2001;115:32–8.
6. Levine RL, Cargile CB, Blazes MS, van Rees B, Kurman RJ, Ellenson LH. PTEN mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma. Cancer Res 1998;58:3254–8.
7. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. Nat Genet 1998;19:348–55.
8. Podsypanina K, Ellenson LH, Nemes A, et al. Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci U S A 1999;96:1563–8.
9. Podsypanina K, Lee RT, Politis C, et al. An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten$^{+/-}$ mice. Proc Natl Acad Sci U S A 2001;98:10320–5.
10. Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mak TW. High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/− mice. Cancer Res 2000;60:3605–11.
11. Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP. Pten and p27^{KIP1} cooperate in prostate cancer tumor suppression in the mouse. Nat Genet 2001;27:222–4.
12. Wang H, Douglas W, Lia M, et al. DNA mismatch repair deficiency accelerates endometrial tumorigenesis in Pten heterozygous mice. Am J Pathol 2002;160:1481–6.
13. Couse JF, Davis VL, Hanson RB, et al. Accelerated onset of uterine tumors in transgenic mice with aberrant expression of the estrogen receptor after neonatal exposure to diethylstilbestrol. Mol Carcinog 1997;19:236–42.
14. Wang HQ, Altomare DA, Skele KL, et al. Positive feedback regulation between AKT activation and fatty acid synthase expression in ovarian carcinoma cells. Oncogene 2005;24:3574–82.
15. Martin MB, Franke TF, Stoica GE, et al. A role for Akt in mediating the estrogenic functions of epidermal growth factor and insulin-like growth factor I. Endocrinology 2000;141:4503–11.
16. Stoica GE, Franke TF, Moroni M, et al. Effect of estradiol on estrogen receptor-alpha gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway. Oncogene 2003;22:7998–8011.
17. Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. J Biol Chem 2001;276:9817–24.
18. Sun M, Paciga JE, Feldman RI, et al. Phosphatidylinositol-3-OH Kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor alpha (ERalpha) via interaction between ERalpha and PI3K. Cancer Res 2001;61:5985–91.
19. Wakeling AE, Dukes M, Bowler J. A potent specific pure antiestrogen with clinical potential. Cancer Res 1991;51:3867–73.
20. Couse JF, Dixon D, Yates M, et al. Estrogen receptor-alpha knockout mice exhibit resistance to the developmental effects of neonatal diethylstilbestrol exposure on the female reproductive tract. Dev Biol 2001;238:224–38.
21. Kimura F, Watanabe J, Hata H, et al. PTEN immunohistochemical expression is suppressed in G_1 endometrioid adenocarcinoma of the uterine corpus. J Cancer Res Clin Oncol 2004;130:161–8.
22. Shoman N, Klassen S, McFadden A, Bickis MG, Torlakovic E, Chibbar R. Reduced PTEN expression predicts relapse in patients with breast carcinoma treated by tamoxifen. Mod Pathol 2005;18:250–9.
23. Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. Mod Pathol 2001;14:672–6.
24. Saal LH, Holm K, Maurer M, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 2005;65:2554–9.
25. Stal O, Perez-Tenorio G, Akerberg L, et al. Akt kinases in breast cancer and the results of adjuvant therapy. Breast Cancer Res 2003;5:R37–44.
26. Panigrahi AR, Pinder SE, Chan SY, Paish EC, Robertson JFR, Ellis IO. The role of PTEN and its signalling pathways, including AKT, in breast cancer; an assessment of relationships with other prognostic factors and with outcome. J Pathol 2004;204:93–100.
27. Rose PG, Brunetto VL, VanLe L, Bell J, Walker JL, Lee RB. A phase II trial of anastrozole in advanced recurrent or persistent endometrial carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol 2000;78:212–6.

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