An activating Pik3ca mutation coupled with Pten loss is sufficient to initiate ovarian tumorigenesis in mice

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Mutations in the gene encoding the p110α subunit of PI3K (PIK3CA) that result in enhanced PI3K activity are frequently observed in human cancers. To better understand the role of mutant PIK3CA in the initiation or progression of tumorigenesis, we generated mice in which a PIK3CA mutation commonly detected in human cancers (the H1047R mutation) could be conditionally knocked into the endogenous Pik3ca locus. Activation of this mutation in the mouse ovary revealed that alone, Pik3caH1047R induced premalignant hyperplasia of the ovarian surface epithelium but no tumors. Concomitantly, we analyzed several human ovarian cancers and found PIK3CA mutations coexistent with KRAS and/or PTEN mutations, raising the possibility that a secondary defect in a co-regulator of PI3K activity may be required for mutant PIK3CA to promote transformation. Consistent with this notion, we found that Pik3caH1047R mutation plus Pten deletion in the mouse ovary led to the development of ovarian serous adenocarcinomas and granulosa cell tumors. Both mutational events were required for early, robust Akt activation. Pharmacological inhibition of PI3K/mTOR in these mice delayed tumor growth and prolonged survival. These results demonstrate that the Pik3caH1047R mutation with loss of Pten is enough to promote ovarian cell transformation and that we have developed a model system for studying possible therapies.

Introduction

In human cancers, somatic mutations in PIK3CA commonly target specific hot spots in the helical (E542 and E545) and kinase (H1047) domains (1, 2), resulting in enhanced lipid kinase activity and upregulation of downstream signaling events such as phosphorylation of Akt (3). Activation of Akt is observed in up to 70% of ovarian cancers (4) due to a variety of causes, including PIK3CA mutations (8%–12%) or amplification (3%–8%) and/or PTEN loss (27%) or mutations (3–8%) (1, 5–10). Loss of Pten (11, 12) or overexpression of activated PI3K in the mouse ovarian surface epithelium (OSE) (13) do not lead to tumor formation; however, the ability of mutant Pik3ca to initiate tumorigenesis or drive tumor progression has not been established. Here we describe an animal model that allows us to study the effects of the Pik3caH1047R mutation in the physiologically relevant context of a somatic, heterozygous Pik3ca mutation expressed at endogenous levels in otherwise normal cells and tissues.

Results and Discussion

We analyzed 87 human ovarian cancers for changes in PI3K pathway genes PIK3CA, PIK3R1 (encoding the PI3K p85 regulatory subunit), PTEN, and also in KRAS, which interacts with PI3K to mediate its activity (refs. 14, 15, and Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI59309DS1). We found 37% of samples exhibited genetic aberrations in one of these genes. Notably, 4 (40%) of the 10 patients who had PIK3CA mutations also exhibited PIK3R1, PTEN, and/or KRAS mutations. Analysis of 34 ovarian cancer cell lines found 3 (IGROv1, TOV21G, and MCAS) had coexisting PIK3CA mutation with Pten and/or KRAS aberrations (Supplemental Table 1). Consistent with this, studies in endometrioid ovarian (11), breast, endometrial, and colon cancers (16) have identified tumors with coexistent PIK3CA and PTEN mutations. Neither the role of endogenous levels of mutant PIK3CA nor the cooperative nature of PIK3CA and PTEN mutations has been evaluated in vivo.

To address this, we used what we believe to be a novel exon-switch strategy to generate a mutant mouse harboring a germline Pik3ca allele with a conditional H1047R mutation. We inserted loxP sites flanking exon 20 of Pik3ca and downstream placed a tandem copy of exon 20 containing a CAT→AGG change in codon 1047 (Figure 1A and Supplemental Figures 1 and 2). Adenoviral Cre–mediated (AdCre-mediated) recombination leads to replacement of wild-type with mutant exon 20, resulting in p110α-H1047R protein expression at endogenous levels in otherwise normal cells, thus accurately reproducing the scenario of a naturally occurring muta-
plasia varied between mice and was highest in the Pik3ca<sup>H1047R</sup> and Pik3ca<sup>H1047R;Pten<sup>del/del</sup></sup> compared with the Pten<sup>del/del</sup> mice, suggesting that alone, Pik3ca<sup>H1047R</sup> more potently promotes proliferative changes compared with Pten loss. In some Pik3ca<sup>H1047R</sup> mice, the hyperplasia resembled epithelial proliferation seen in the borderline (low malignant potential) subtype of human ovarian serous carcinoma. In 2 mice, we observed microinvasion of the ovarian stroma, indicating that the epithelial proliferation was neoplastic despite the absence of definitive cytological evidence of malignancy. The inability of Pik3ca<sup>H1047R</sup> to induce ovarian tumorigenesis differs from transgenic mouse models of Pik3ca<sup>H1047R</sup> inducing lung and breast (20–23) tumors, but these models are driven by overexpressed Pik3ca<sup>H1047R</sup> rather than mutation of the endogenous gene, as occurs in human tumors. Possibly, the ovary could be less susceptible to transformation via a single mutation compared with other tissue types. Studies are underway to examine the role of this mutation in the lung, breast, and gastrointestinal tract.

In contrast to the single mutant mice, all Pik3ca<sup>H1047R;Pten<sup>del/del</sup></sup> mice developed ovarian masses, necessitating sacrifice with a median latency of 16 weeks (Figure 2B and Figure 3, A and B). Macroscopic analyses at autopsy revealed that many Pik3ca<sup>H1047R;Pten<sup>del/del</sup></sup> mice developed hemorrhagic ascites, and some showed intra-peritoneal masses on the peritoneal wall and diaphragm. No distant metastases were evident macroscopically. Histological examination confirmed malignancy in 15 of 18 ovarian masses, consisting of ovarian serous adenocarcinomas (Figure 3, D and E), ovarian granulosa cell tumors (Figure 3, I–K), and a single ovarian luteoma (Table 1). Three mice had collision tumors composed of ovarian serous adenocarcinomas (Figure 3, D and E), consisting of ovarian serous adenocarcinomas (Figure 3, D and E), and gastrointestinal tract.

The inability of the Pik3ca<sup>H1047R</sup> mutation to promote proliferative changes in these mouse models offers an explanation for the much lower malignancy rate in humans compared with mouse models. These mouse models offer a powerful tool for investigating the role of PIK3CA amplification and mutation in human ovarian carcinomas.
can cooperate to potently promote proliferation or transformation in several cell types, including OSE, ovarian granulosa cells, fibroblasts, and smooth muscle cells following AdCre infection.

We compared PI3K/Akt/mTOR pathway signaling in Pik3ca<sup>H1047R</sup> Pten<sup>del/del</sup> tumor lysates with that in other mouse ovarian cancer models: Kras<sup>G12D</sup> Pten<sup>del/del</sup> and Apc<sup>S580/S580</sup> Pten<sup>del/del</sup> (refs. 11, 12, and Figure 3L). Pik3ca<sup>H1047R</sup> Pten<sup>del/del</sup> and Kras<sup>G12D</sup> Pten<sup>del/del</sup> tumors exhibited equivalent pathway signaling to multiple substrate proteins. While Apc<sup>S580/S580</sup> Pten<sup>del/del</sup> tumors showed equivalent p-Pdk1 and p-Rps6 levels, they showed relatively low levels of p-Akt and its substrates. This suggests that cooperating mutations — Pten loss and Kras or Pik3ca mutation, but not Apc mutation — are required to robustly activate Akt and its direct targets.

These mutations may cooperate because both Pik3ca<sup>H1047R</sup> kinase activity and loss of PTEN's phosphatase activity are necessary to reach a threshold of PIP<sub>3</sub> levels required for transformation. Alternatively, since PTEN has additional, PI3K-independent tumor-suppressive functions (25), its loss may promote tumorigenesis via these additional mechanisms. Finally, both mutations may be required to overcome negative feedback loops that could be activated as a result of only one of these genetic events.

To examine whether established Pik3ca<sup>H1047R</sup> Pten<sup>del/del</sup> tumors remained dependent on PI3K pathway activity for tumor maintenance, we utilized an ATP-competitive PI3K/mTOR inhibitor, PF04691502, currently in phase I clinical trials (26, 27). Treatment of Pik3ca<sup>H1047R</sup> Pten<sup>del/del</sup> mice commenced 10 weeks following AdCre exposure (Figure 3M), and tumor progression was monitored by ultrasound (Supplemental Figure 8). Early response was characterized by cytostasis, which significantly lengthened median survival time (Figure 3M), indicating a continued dependence on PI3K and/or mTOR for tumorigenesis in this model. Interestingly, tumors eventually regrew despite continuous PI3K/mTOR inhibition with PF04691502. This suggests that patients with PIK3CA<sup>H1047R</sup> mutations and loss of PTEN could be initially responsive to PI3K/mTOR inhibition, but that resistance is likely to develop. This model has the potential to provide a crucial new system for examining resistance mechanisms to PI3K pathway inhibitors and for testing novel therapeutics targeting the PI3K pathway alone or in combination with other therapies.

In summary, study of the Pik3ca<sup>H1047R</sup> mouse model described herein has demonstrated that Pik3ca mutation requires a second hit to initiate tumorigenesis in the ovary. We found that mutations in PI3K regulatory proteins co-occur in human ovarian tumors with PIK3CA mutations and demonstrate that in vivo, Pik3ca<sup>H1047R</sup> and loss of Pten is sufficient to promote ovarian cell transformation.
Methods

Generation of Pik3ca<sup>H1047R</sup> and Pten<sup>del/del</sup> mice. Pik3ca<sup>H1047R</sup> mice were generated by homologous recombination in embryonic stem cells (Ozgene). The targeting construct, detailed in Supplemental Figure 1, was assembled from C57BL/6 genomic DNA and cloned into the Ozgene F_PacI_Neo vector containing a PGK-neomycin cassette flanked by FLP recombinase target (FRT) sequences. The targeting vector was electroporated into 129S1/Sv-derived W9.5 ES cells. Targeted ES cells were injected into C57BL/6 blastocysts and chimeras crossed with C57BL/6 mice. The PGK-neomycin cassette was deleted by crossing with C57BL/6 ACTB-FLPe mice.

AdCre recombination in the ovary. Cells in the ovarian bursa were infected with AdCre, a gift from Walter Thomas (Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia) as described previously (18); details are provided in Supplemental Methods. Endpoints were excessive tumor burden (>1.5 cm<sup>3</sup>), severe abdominal distension, or signs of severe illness (hunching, ruffled fur, or loss of responsiveness).

Mouse cohorts. Pten<sup>del/del</sup> mice (c;129S4-Pten<sup>tm1Hwu</sup>/J) were from The Jackson Laboratory. All Pik3ca<sup>H1047R</sup>Pten<sup>del/del</sup> mice were maintained on a mixed (C57BL/6; BALB/c; 129S4) background, and littermates were used where possible. Pik3ca<sup>H1047R</sup> refers to heterozygotes.

Figure 3

Pik3ca<sup>H1047R</sup> and Pten<sup>del/del</sup> cooperate to promote tumorigenesis in mouse ovary. (A) Kaplan-Meier curve of Pik3ca<sup>H1047R</sup>Pten<sup>del/del</sup> mice (red; median survival, 16 weeks) compared with control mice (n in parentheses) exposed to AdCre in the ovarian bursa. (B) Ovarian tumors from Pik3ca<sup>H1047R</sup>Pten<sup>del/del</sup> mice (20–25 weeks after AdCre infection) and non-tumor-bearing control mice 1 year after AdCre infection. Scale bars: 1 cm. (C–K) Representative histopathology of tumors from Pik3ca<sup>H1047R</sup>Pten<sup>del/del</sup> mice. Non-AdCre-exposed ovary (C), serous adenocarcinoma (D–H), and granulosa cell tumor (I–K). Staining with H&E (C–E, I, and J), p-Akt S473 (F, high-power inset), p-Rps6 (G, high power inset), pan-keratin (H, adjacent uterus inset), or inhibin (K) is shown. Scale bars: bracketed, 500 mm; unbracketed, 50 mm. B, bursa; CL, corpus luteum; F, follicle; FP, fat pad. (L) Protein blots from Kras<sup>G12D</sup>Pten<sup>del/del</sup> (Pten Kras), Pik3ca<sup>H1047R</sup>Pten<sup>del/del</sup> (Pten Pik3ca), and Apc<sup>S580/S580</sup>Pten<sup>del/del</sup> (Pten Apc) ovarian tumors. (M) Tumor-bearing Pik3ca<sup>H1047R</sup>Pten<sup>del/del</sup> mice were treated with vehicle or PF04691502 daily. Mice receiving vehicle exhibited 4.5 weeks median survival compared with 11 weeks in PF04691502-treated mice; log-rank (Mantel-Cox) test, P = 0.0006.

Histology and protein analysis. Tumors were formalin fixed, and immunohistochemistry was performed as described previously (27). For Western blotting, tumors were snap frozen and lysed in RIPA buffer. Antibodies are detailed in Supplemental Methods.

In vivo therapy. Mice received vehicle (0.5% methylcellulose) or 10 mg/kg PF04691502 orally, daily. Ovary volume was monitored by ultrasound imaging (Vevo 770, Visualsonics). Endpoints were as above or weight loss of greater than 20%.
Table 1

<table>
<thead>
<tr>
<th>Histology by genotype</th>
<th>Frequency</th>
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<tr>
<td>Pik3caH1047R or Pten deletion in the ovary induces serous papillary hyperplasia and together can cooperate to induce serous adenocarcinomas or granulosa cell tumors</td>
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<tr>
<td>Pik3caH1047R/Pten+/−</td>
<td>Normal 6/6 (100%)</td>
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<tr>
<td></td>
<td>SPH grade 1 1/5 (20%)</td>
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<td></td>
<td>SPH grade 2 3/5 (60%)</td>
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<td></td>
<td>SPH grade 1 1/5 (20%)</td>
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<tr>
<td>Pik3caH1047R/Pten+/−</td>
<td>Normal 3/3 (100%)</td>
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<td></td>
<td>SPH grade 1 1/5 (20%)</td>
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<td></td>
<td>SPH grade 2 1/5 (20%)</td>
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<td></td>
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<td>SPH grade 1 5/6 (83.3%)</td>
</tr>
<tr>
<td></td>
<td>Normal 6/6 (100%)</td>
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*One mouse was sacrificed due to illness at 14 weeks (unknown cause); ovaries were described as normal. Collision tumors (both serous and granulosa cell histopathology) were observed in 3 mice. SPH, serous papillary hyperplasia. Mice were analyzed at 45–52 weeks after AdCre infection, except for the Pik3caH1047R/Pten−/− group, which were sacrificed at 9–28 weeks after AdCre infection due to illness.

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Statistics. Statistical analyses were performed using a 2-tailed Student’s t test or log-rank (Mantel-Cox) Kaplan-Meier survival test. P values less than 0.05 were considered statistically significant.

Study approval. Animal experiments followed the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the PMCC ethics committee.