Prolactin Activates ERα in the Absence of Ligand in Female Mammary Development and Carcinogenesis in Vivo

Kathleen A. O’Leary, Fatou Jallow, Debra E. Rugowski, Ruth Sullivan, Kerstin W. Sinkevicius, Geoffrey L. Greene, and Linda A. Schuler


Resistance of estrogen receptor positive (ERα+) breast cancers to antiestrogens is a major factor in the mortality of this disease. Although activation of ERα in the absence of ligand is hypothesized to contribute to this resistance, the potency of this mechanism in vivo is not clear. Epidemiologic studies have strongly linked prolactin (PRL) to both development of ERα+ breast cancer and resistance to endocrine therapies. Here we employed genetically modified mouse models to examine the ability of PRL and cross talk with TGFβ to activate ERα, using a mutated ERα, ERα(G525L), which is refractory to endogenous estrogens. We demonstrate that PRL promotes pubertal ERα-dependent mammary ductal elongation and gene expression in the absence of estrogen, which are abrogated by the antiestrogen, ICI 182,780 (ICI). PRL and TGFβ together reduce sensitivity to estrogen, and 30% of their combined stimulation of ductal proliferation is inhibited by ICI, implicating ligand-independent activation of ERα as a component of their interaction. However, PRL/TGFβ-induced heterogeneous ERα+ tumors developed more rapidly in the presence of ICI and contained altered transcripts for surface markers associated with epithelial subpopulations and increased signal transducer and activator of transcription 5b expression. Together, these data support strong interactions between PRL and estrogen on multiple levels. Ligand-independent activation of ERα suggests that PRL may contribute to resistance to antiestrogen therapies. However, these studies also underscore ERα-mediated moderation of tumor phenotype. In light of the high expression of PRL receptors in ERα+ cancers, understanding the actions of PRL and cross talk with other oncogenic factors and ERα itself has important implications for therapeutic strategies. (Endocrinology 154: 4483–4492, 2013)
important implications for both understanding PRL actions and identifying therapeutic targets in breast cancer.

We have developed the neu-related-lipocalin (NRL)-PRL transgenic mouse as a preclinical model to examine the actions of PRL in this disease (15, 16). The mammary epithelia of these mice express PRL, elevating local exposure similar to that observed in women (17, 18). Consistent with the epidemiologic studies, nulliparous NRL-PRL females develop diverse aggressive carcinomas that resemble the luminal subtype of clinical breast cancer (19). Despite ERα expression, advanced tumors do not respond to ovariectomy or supplemental 17β-estradiol (19). Transgenic PRL elevates mammary pErk1/2 and pAkt (20), signaling cascades that are associated with phosphorylation of the N-terminal activation function 1 (AF-1) domain of ERα and subsequent activation even in the absence of estrogenic ligands (21–24). PRL also can induce phosphorylation of ERα in human breast cancer cell lines in vitro (25, 26) and induces recruitment of ERα to target genes (27). These observations of PRL actions resemble those reported for growth factors that are also linked to resistance to estrogen-directed therapies for breast cancer (2, 3). However, whether this mechanism plays a role in PRL action in the dynamic in vivo environment is unclear.

PRL also strongly potentiates growth factor signals in vitro and in vivo. In breast cancer cell lines, PRL cooperates with IGF-1 and epidermal growth factor (EGF) receptor ligands to prolong signaling through the ERK1/2 and AKT pathways, by modulation of the trafficking of the respective growth factor receptors (26, 28–31). PRL can also initiate phosphorylation of human epidermal growth factor receptor 2 (32). Many of the carcinomas that develop in NRL-PRL females in vivo express high growth factor receptor 2 (32). Many of the carcinomas that develop in NRL-PRL females in vivo express high levels of erbB family receptors (33). ErbB signals play important roles in normal mammary development as well as breast cancer (34, 35). Transgenic mammary expression of the EGF receptor (EGFR) ligand, TGFα, in combination with PRL, dramatically reduces tumor latency (28). Strikingly, the combination of these 2 transgenes also abrogates the proliferative response of morphologically normal ducts to estrogen, as well as the interaction of estrogen and TGFα in tumorigenesis (26). Although these observations indicate that the interaction of PRL and TGFα confers independence from estrogenic ligands for proliferation, they do not address a role for ERα itself.

In order to address the role of ligand-independent activation of ERα, we have taken advantage of the genetically modified mouse expressing ERα(G525L) (36). This mutated ERα is refractory to 17β-estradiol but retains responsiveness to the synthetic ERα agonist, propylpypyrrozoletriol (PPT) and responds to phosphorylation, permitting analysis of ligand-independent activation. Transgenic mammary PRL and TGFα expression, in conjunction with the antiestrogen ICI 182,780 (ICI), permit dissociation of these factors and estrogen, which is otherwise complicated by estrogenic regulation of the PRL and TGFα promoters (37, 38). In combination, these mouse models enabled us to examine the ability of PRL to activate ERα in the absence of ligand and determine whether this leads to functionally important physiologic and pathologic consequences. Our findings demonstrate significant relevance of this mechanism in vivo and reveal the consequences of ER signals in PRL-TGFα cross talk in mammary tumorigenesis.

Materials and Methods

Reagents

The following antibodies were used for immunohistochemistry: Ki-67 (catalog no. 15580) from Abcam, ERα (sc-542), signal transducer and activator of transcription (Stat)5α (sc-1081), and Stat5b (sc-1656) from Santa Cruz Biotechnology, Inc. PPT was obtained from Orbiter Research, LCC. ICI (Fulvestrant) was obtained from AstraZeneca.

Mice

NRL-PRL [line 1647–13, TgN[Nrl-Prl]23EPS] and NRL-TGFα [line 1385–7, TgN[Nrl-tgfa]25EPS] mice, were generated in the FVB/N strain background as described elsewhere (15, 39). The NRL promoter directs expression to mammary epithelial cells and is not affected by PRL or estrogen (15, 39). Mice expressing a mutated ERα [ERα(G525L)], which is not activated by 17β-estradiol, were derived and maintained on a mixed background, as described elsewhere (36). For experiments examining tumorigenesis, ERα(G525L) mice were backcrossed 5 generations (N5) onto the FVB/N strain. Tail biopsies were collected at weaning, and offspring were screened for the genetic modifications by PCR (primer sequences in Supplemental Table 1 published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Mice were fed Teklad Global Extruded Rodent Diet (soy protein free) ad libitum (Harlan Laboratories). For studies of tumorigenesis, animals were observed weekly for tumors and considered to be end stage when tumor diameter reached 1.5 cm (defined as tumor latency) or 9 months of age. Mice were housed and handled in accordance with the Guide for Care and Use of Laboratory Animals in AAALAC-accredited facilities. All procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee.

Manipulation of estrogen activity

To observe the role of ER-mediated signals in pubertal elongation, females were treated with vehicle, or the ERα agonist, PPT (10 mg/kg), every fourth day starting at day 10 of age. One cohort also was treated with the ER antagonist, ICI (167 mg/kg/wk sc), beginning at 3 weeks of age. Glands were examined at 12 weeks of age. To observe the role of ER-mediated signals in tumorigenesis, females were treated with the ERα agonist, PPT (10 mg/kg), every fourth day starting at day 10 of age until ductal...
elargonation was complete (10 weeks of age), after which one cohort was treated with ICI until 9 months of age.

**Analysis of ductal elongation**

In order to examine the effect of locally elevated PRL on pubertal ductal elongation in the context of the homozygous ERα(G525L) mutation, nontransgenic and NRL-PRL females with wild-type or mutant ERα(G525L) were examined (F2 generation, of the same mixed genetic background). Fourth inguinal mammary glands were fixed in 10% neutral buffered formalin overnight and stored in 70% ethanol. Whole mounts were stained with carmin, dehydrated with graded ethanol, and stored in glycerol until analysis. Ductal elongation was measured by dividing the area of ductal growth (length × width) by the area of the mammary fat pad (length × width).

**Histologic examination of mammary tissue**

Mammary glands were fixed in 10% neutral buffered formalin overnight, embedded in paraffin, and cut into 6-μm sections. Morphologic analysis was performed on hematoxylin and eosiin-stained slides. Ductal proliferation indices were determined by evaluating nuclear Ki67 staining in 1000 epithelial cells from at least 10 morphologically normal ducts. Tumors were classified as ERα+ if more than 10% of the cells displayed nuclear ERα staining. Nuclear Stat5a/b labeling indices were determined by counting 100 tumor cells in 10 random microscopic fields by an investigator blind to the experimental group.

**Real-time quantitative PCR**

Total cellular RNA was isolated from whole mammary glands and tumors using RNeasy Kits (Qiagen, Inc.). For some experiments, poly(A)+ RNA was purified using the Ambion MicroPoly(A) Purist Kit (Life Technologies). cDNA was synthesized and quantitative real time-PCRs were carried out as described elsewhere (19). Primers used are shown in Supplemental Table 1.

**Statistical analyses**

Statistical analyses were performed using Prism v.5 (GraphPad Software, Inc.). Differences were considered significant at P < .05.

**Results**

**PRL induces ERα-dependent ductal elongation in the absence of ligand**

At puberty, ovarian estrogens initiate the extension of the rudimentary mammary ductal tree throughout the mammary fat pad, a process that has been shown to be dependent on epithelial ERα (40, 41). By 12 weeks of age, the ducts have elongated to fill nearly the entire fat pad, as observed in whole mounts of inguinal glands of nontransgenic and NRL-PRL females expressing wild-type ERα (89 ± 11%, 91 ± 11%, respectively). We used this physiologic event as one endpoint to determine whether PRL can activate ERα without estrogenic ligand. As shown in Figure 1, mammary glands of nontransgenic females that were homozygous for the ERα(G525L) mutation exhibited minimal ductal development, which was increased about 6-fold by administration of the synthetic ERα-selective agonist, PPT, as previously reported (36). Although elevated local PRL did not stimulate ductal elongation to the same extent as PPT, it did increase ductal development about 4-fold, which was reduced to baseline levels by ICI. Notably, PRL and PPT in combination strikingly promoted ductal elongation.

The ability of PRL to promote ERα-dependent ductal elongation in the absence of estrogenic ligand was reflected in transcript levels for cytokeratins expressed by both basal (Krt5) and luminal (Krt18,19) epithelial cells (42) (Figure 2A). Transcripts for other direct and indirect gene targets of estrogen at puberty (43, 44) were also elevated by locally increased PRL and reduced to levels not significantly different from ERα(G525L) females by ICI, including mRNAs for the other components of the ERα-regulatory network, Gata3 and FoxA1, the putative mediator of estrogen action in ductal elongation, amphiregulin, and other estrogen targets (Figure 2B). In contrast, mRNAs for ERα, and IGF-1 and -2 were not altered by genotype or treatment, consistent with their expression in stroma as well as epithelia (Supplemental Figure 1). Together, these data indicate that PRL can activate ERα in...
the absence of ligand to functionally promote ductal elongation.

PRL/TGFα drives epithelial proliferation partially via ligand-independent activation of ERα

The apparent insensitivity of mammary epithelial proliferation and tumorigenesis to estrogen ligand in NRL-PRL/TGFα females (26) suggested that ligand-independent activation of ERα may play an important role in the subsequent pathology. To minimize complexities from the mixed genetic background for these studies, we back-crossed the ERα(G525L) mutation into the FVB/N strain.

In order to expand the mammary epithelial subpopulation, females of all genotypes were treated with PPT until 10 weeks of age to mimic the effect of pubertal estrogen on ductal elongation. Thereafter, NRL-PRL/TGFα females with wild-type ERα or ERα(G525L) were assigned to vehicle or ICI treatments. ICI markedly reduced uterine weight (Supplemental Figure 2), demonstrating the efficacy of this treatment.

At 9 months of age or end stage, mammary glands were examined. Proliferation of morphologically normal luminal epithelium was assessed using Ki67. As previously reported (28), exposure to locally elevated PRL and TGFα dramatically increased proliferation of mammary ductal epithelia (Figure 3B and Supplemental Figure 3). Consistent with the observed independence from ovarian estrogen (26), luminal epithelia in NRL-PRL/TGFα/ERα(G525L) females proliferated at the same rate as that in NRL-PRL/TGFα females with wild-type ERα. Treatment of both genotypes with ICI significantly decreased proliferation. However, despite this inhibition of ER-mediated signals, epithelial proliferation remained 3 times higher than in wild-type glands. These data indicate that a significant portion, but not all, of PRL/TGFα-stimulated proliferation is mediated through ER.

In the absence of ER-mediated signals, PRL/TGFα-promoted tumors develop more rapidly and exhibit a shift in histotype

In contrast to the inhibitory effect of ICI on proliferation of morphologically normal epithelium, ICI treatment accelerated tumorigenesis in NRL-PRL/TGFα females (Figure 4A). One hundred percent of NRL-PRL/TGFα females with wild-type ERα developed large mammary tumors with a latency similar to previous reports (26, 28), which was significantly reduced by ICI treatment (P < .05). The ERα(G525L) mutation increased the latency (P < .01) and decreased the incidence of mammary tumors (P < .05), compared with tumors from females with wild-type ERα, perhaps reflecting the smaller epithelial popu-
lation (Supplemental Figure 4) and/or ovarian dysfunction in these animals (45). However, ICI treatment increased the number of tumors in the NRL-PRL/TGFα/ERα(G525L) females compared with that in control NRL-PRL/TGFα/ERα(G525L) females ($P < .05$). These data indicate that ICI exerts protumorigenic actions in this model.

Tumors that developed in all genotypes, regardless of ICI treatment, were very heterogeneous and displayed many shared features (Table 1 and Figure 5). In contrast to the complex macrocystic tumors that developed in NRL-PRL/TGFα females on a standard diet (28), the tumors that developed on the soy-free diet in the current study were largely solid, with occasional restricted areas of complex microcysts in some tumors (Figure 5C). Limited studies indicated that the tumors were transplantable. Despite the shared elements, however, significant differences with genotype and ICI treatment were observed (Table 1). Tumors from females with wild-type ERα without ICI treatment were predominantly glandular with microacinar structures, many of which contained copious secretions (Figure 5A). In contrast, tumors from these females treated with ICI or carrying the mutant ERα(G525L) showed a significant shift to tumors that exhibited predominantly papillary histotypes (Figure 5B; $P < .05$, Fisher’s exact test). Many of the tumors, especially the NRL-PRL/TGFα females without ICI treatment, contained large lipid droplets, consistent with the action of estrogen on metabolism (43). Finally, a few of the tumors contained small squamous regions (Figure 5D). Of note, all tumors in all genotypes and treatments, including those that developed in the presence of postpubertal ICI, displayed high levels of ERα expression (Table 1 and Figure 5, E and F), despite the inhibition of ER signals (Supplemental Figure 2). In contrast, PRL/TGFα-induced tumors expressed little or no detectable progesterone receptor (PgR), similar to our previous study (26).

To further investigate the role of estrogen signals in tumorigenesis induced by PRL/TGFα, we examined the effect of postpubertal ICI on select transcripts in NRL-PRL/TGFα tumors by quantitative real time-PCR. As expected, tumors that developed with chronic inhibition of ER signals displayed higher levels of ERα and lower levels of PgR mRNA, consistent with the ability of estrogen to inhibit ERα transcription (46) and stimulate PgR transcription (47) (Figure 6A). mRNA for ERβ was not detectable in these tumors, in contrast to abundant transcripts in the uterus and ovary (data not shown). Levels of Ki67 and survivin mRNAs did not reveal significant differences (data not shown). Although levels of mRNA for Krt5 and Krt8, markers for basal and luminal cells, respectively, and the transcription factor, Gata3, were not altered by ICI (Figure 6B), transcript levels of integrins associated with various epithelial subpopulations exhibited significant differences: ICI decreased transcripts for β1-integrin ($P < .04$) and increased those for α6-integrin ($P < .03$) (Figure 6C). Moreover, tumors from animals treated with ICI had significantly higher levels of Stat5b transcripts (Figure 6D; $P = .007$) and trended to have lower levels of Stat5a mRNA ($P = .06$).

In light of the central role that Stat5a plays in the mediation of the physiological signals of PRL (48), and high expression of Stat5b in many breast cancers (48–51), we further examined expression of these proteins in tumors induced by PRL/TGFα in the presence and absence of ICI. As shown in Figure 7, tumors that developed in the presence of ICI contained a greater number of cells containing nuclear Stat5b ($P < .01$) and fewer cells with nuclear Stat5 ($P < .01$) than tumors that developed in the absence of ICI.

![Figure 4](https://academic.oup.com/endo/article-abstract/154/12/4483/2433333/4483)
Discussion

ERα expression is the foremost prognostic and treatment indicator in breast cancer. Nonetheless, more than 25% of ERα+ cancers fail to respond to antiestrogen treatments (1). The interactions between PRL and estrogen in normal physiology and the strong association of PRL with the risk, progression, and therapeutic responsiveness of ERα+ breast cancer stress the importance of understanding the role of PRL in this cancer subtype. Ligand-independent activation of ERα has been suggested as one mechanism whereby tumors escape endocrine treatments (2–4). However, although evidence from our laboratory and others implicates PRL action by this mechanism in breast cancer cells in vitro (25–27), the potency of this mechanism in vivo has not been demonstrated. The studies described herein demonstrate that PRL can activate ERα.

Table 1. Prominent Histologic Features of Tumors

<table>
<thead>
<tr>
<th>Genotype/Treatment</th>
<th>Glandular/Microacinar</th>
<th>Microcystic</th>
<th>Papillary</th>
<th>ERα+</th>
</tr>
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<tr>
<td>PRL/TGFα</td>
<td>11/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5/11</td>
<td>2/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11/11</td>
</tr>
<tr>
<td>PRL/TGFα + ICI</td>
<td>11/13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9/13</td>
<td>6/13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13/13</td>
</tr>
<tr>
<td>PRL/TGFα/ERα(G525L)</td>
<td>6/10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7/10</td>
<td>7/10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10/10</td>
</tr>
<tr>
<td>PRL/TGFα/ERα(G525L)+ICI</td>
<td>5/9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6/9</td>
<td>6/9&lt;sup&gt;b&lt;/sup&gt;</td>
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Tumors were highly heterogeneous, containing multiple different regions of distinct histotypes. More than 10% of tumor cells with nuclear ERα staining were considered to be ERα+. Different letters denote statistical differences among groups (P < 0.05; Fisher’s exact test).

**Figure 5.** Tumors from different genotypes/treatments are heterogeneous, sharing some but not all histologic features. A, glandular region containing prominent lipid droplets in tumor from a NRL-PRL/TGFα female. B, papillary region of tumor from a NRL-PRL/TGFα + ICI female. C, Cystic region of tumor from a NRL-PRL/TGFα + ICI female. D, Squamous differentiation with lamellar keratin accumulation in tumor from a NRL-PRL/TGFα/ERα(G525L) female. E, ERα staining of a glandular tumor from a NRL-PRL/TGFα female. F, ERα staining of papillary tumor from a NRL-PRL/TGFα + ICI female. Original magnification, A–C, E, and F ×100; D, ×200. Scale bars, 100 μm.

**Figure 6.** Chronic postpubertal inhibition of ER-mediated signals with ICI alters select transcripts in end-stage mammary tumors in NRL-PRL/TGFα females. Specific transcripts were quantitated in total RNA by qRT-PCR, normalized to 18S RNA. (−ICI, open bars; +ICI, shaded bars). Mean ± SEM; n = 5–6. Significant differences were determined by Student’s t test (*, P < .05; **, P < .01).
in the absence of estrogenic ligand in vivo, driving ERα-dependent ductal elongation and gene expression at puberty. Moreover, they show that the cooperative stimulation of epithelial proliferation by PRL and the EGFR ligand, TGFα, is partially mediated by ER, independent of estrogen ligand. However, inhibition of postpubertal ER signals accelerates tumorigenesis and shifts the histotype of the resulting tumors, despite high levels of ERα expression.

The ability of locally elevated PRL to drive ERα-dependent ductal elongation (40, 41) in NRL-PRL/ERα(G525L) females demonstrates the efficacy of PRL-induced ligand-independent activation of ERα in vivo. The cooperation between PRL and the estrogen agonist, PPT, in ductal development likely reflects the multiple levels of cross talk between these hormones in mammary epithelial cells, including increasing expression of the other’s receptors (52–54) and cross talk to tethered ERα-mediated signals, such as AP-1 (55, 56), in addition to PRL-induced activation of the AF-1 domain of ERα (25–27).

Our findings are consistent with the ability of supplemental 17β-estradiol to cooperate with transgenic PRL in mammary tumorigenesis (20). The increased efficacy of an aromatase inhibitor and PRLR-neutralizing antibody to reduce growth of 7,12-dimethylbenz(a)anthracene-induced ERα+ rat mammary tumors (57) indicates that the potency of this interaction may be exploited to enhance therapeutic strategies.

Cross talk between PRL and the EGFR ligand, TGFα, abrogates mitogenic responsiveness of morphologically normal mammary epithelial cells to 17β-estradiol (26), confirmed here by the failure of the ERα(G525L) mutant to alter ductal proliferation in these animals. Notably, it is the combination of the PRL and TGFα transgenes that confers this resistance; proliferation of ductal epithelium in single transgenic NRL-PRL and NRL-TGFα females is highly estrogen responsive (26). Despite this insensitivity to ligand, our findings here demonstrate that ER-dependent signals mediate about one third of PRL/TGFα-induced proliferation. Our results are consistent with the strong cross talk of these hormones/cytokines to signaling cascades that lead to phosphorylation of ERα at residues in the AF-1 domain associated with activation in the absence of ligand (21–24, 26, 28–31). Development of antibodies that recognize modifications of murine ER will assist in understanding the scope of posttranslational modifications initiated by PRL and TGFα individually and in combination, which result in insensitivity to ligand as well as ligand-independent activation. Strikingly, however, even in the absence of ER-mediated signals, PRL/TGFα cross talk still elevates proliferation more than 3-fold above that observed in wild-type ducts, underscoring the strength of their interaction in disease processes.

PRL/TGFα-driven tumorigenesis in this model resulted in strongly ERα+ tumors, regardless of ICI treatment, indicating that postpubertal ER-mediated signals are not necessary for luminal tumorigenesis. These findings support a minimal contribution of estrogen to ERα+ breast cancer, as suggested by the epidemiologic data indicating increased ERα+ tumors after menopause (58, 59). However, in contrast to the positive contribution of ER-mediated signals to the proliferation of morphologically normal ductal epithelium in NRL-PRL/TGFα females, postpubertal inhibition of ER signals accelerated tumorigenesis. Moreover, tumors that developed with chronic inhibition of ER-mediated signals had higher levels of transcripts for α6-integrin and lower levels of β1-integrin, a combination found in adult mammary stem cells (60) and a stem cell-like subpopulation of MCF-7 cells (61). These findings mimic the clinical link between weak ex-
pression of estrogen-driven genes and poor outcomes, features of the luminal B breast cancer subtype that frequently are attributed to heightened growth factor activity (62, 63). Interestingly, stable expression of ERα(S118A) or ERα(S167A) in MCF7 cells, both likely targets of the robust cross talk of TGFα and PRL to ERK1/2 and AKT in these cells (25, 26, 28), increases growth and invasiveness (64). Together with the literature, our data support a model of ER-driven differentiated behaviors, despite the mitogenic effects of these signals.

Interestingly, ER-mediated signals modulated the balance of Stat5a/Stat5b expression. Stat5a is the major Stat5 isoform expressed physiologically in the mammary gland, but Stat5b is also highly expressed in many breast cancers (48–51). Although control of the relative levels of the Stat5 isoforms is not well understood, acute estrogen treatment of mice increases mammary Stat5a mRNA (65), consistent with our data. PRL can activate both Stat5 isoforms, but Stat5a is the best-characterized mediator of PRL actions during pregnancy (48, 49, 66). Growth factors can activate Stat5b via kinases that are also nodes of signaling cross talk with PRL (67, 68). In part because of a shared amino acid sequence surrounding Tyr 694/699, a phosphorylated residue necessary for dimerization and a shared amino acid sequence surrounding Tyr 694/699, a phosphorylated residue necessary for dimerization and nuclear translocation (69), many studies have not distinguished these isoforms. However, it was reported recently that reduced nuclear Stat5a, but not Stat5b, is associated with higher risk of resistance to antiestrogen therapies and worse outcomes (49). Multiple studies have demonstrated the complex relationship between estrogen and Stat5 signals, with disparate findings, depending on experimental design and readout (67). Interestingly, activated ERα suppresses EGF signals to Stat5b in breast cancer cells in vitro (70), suggesting that ICI may strengthen Stat5b-mediated signals in our model. Furthermore, the Src-EGFR-Stat5b cascade can induce tamoxifen resistance (71). In support of the clinical findings demonstrating distinct roles for the Stat5 isoforms, accumulating studies demonstrate functional differences (72, 73) and a large subset of differentially regulated genes (49). Notably, Stat5b, but not Stat5a, mediates PRL-induced α6-integrin mRNA in MCF-7 cells (49), consistent with our findings. Together, our studies provide insight into the network of interactions among PRL, growth factors, and estrogen in the complex in vivo environment and provide a robust model for further investigation.

In summary, our data extend our understanding of the synergistic cross talk between PRL and estrogen, revealing ligand-independent activation of ERα as an additional underlying mechanism, which may impact both normal mammary function as well as pathology. Our findings underscore the ability of PRL to promote luminal breast cancer and its strong cooperation with growth factors in oncogenesis and point to the contributions of PRL to the intricate cross talk between growth factors and estrogen signals, including altered responsiveness to estrogen ligand. These studies support the potential for therapeutics that target PRL alone or in combination with estrogen- or growth factor-directed therapies. However, the altered phenotype and accelerated development of the tumors that develop in the presence of the antiestrogen underscore the importance of validated biomarkers for treatment selection.

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Address all correspondence and requests for reprints to: Linda A. Schuler, Department of Comparative Biosciences, 2015 Linden Drive, University of Wisconsin-Madison, Madison, WI 53706. E-mail: schulerl@svm.vetmed.wisc.edu.

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Current address for K.W.S.: Children’s Hospital Boston, Harvard University, Boston, MA 02115.

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