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The Chemopreventive Effect of Mifepristone on Mammary Tumorigenesis is Associated with an Anti-invasive and Anti-inflammatory Gene Signature

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Abstract

Progesterone receptor (PR) antagonists are potent antitumor agents in carcinogen and progestin-dependent mammary tumorigenesis models through both PR and non-PR-mediated mechanisms. The PR antagonist mifepristone/RU486 has been used primarily as an abortifacient possessing high affinity for both the PR and glucocorticoid receptors (GR). To determine whether mifepristone would be effective as a chemopreventive agent, we assessed its effect on progestin/DMBA-induced mammary carcinogenesis in wild-type (WT) and estrogen receptor- α -positive (ER+) transgenic mice expressing the dominant-negative Pax8PPAR γ (Pax8) fusion protein. Mifepristone administered at a dose of 2.5 mg significantly delayed mammary tumorigenesis in WT, but not in Pax8 mice, whereas, a three-fold higher dose almost completely blocked tumorigenesis in both WT and Pax8 mice. The sensitivity of WT mice to 2.5 mg mifepristone correlated with a expression profile of 79 genes in tumors, 52 of which exhibited the opposite response in Pax8 mice, and corresponded primarily to the down-regulation of genes associated with metabolism, inflammation and invasion. These results suggest that the chemopreventive activity of mifepristone in WT mice correlates with a specific gene expression signature that is associated with multiple nuclear receptor signaling pathways.

Introduction

Progesterone is a major physiological regulator of reproduction through the mammary gland, uterus, ovary, and hypothalamic-pituitary axis (1), and its interaction with the progesterone receptor (PR) is essential for lobulo-alveolar development of the mammary gland (2). Although its physiological function is complex, the PR is believed to drive proliferation of mammary epithelial cells in two waves, the first requiring PR+ cells, and the second wave comprising mostly PR-cells by a paracrine mechanism involving RANKL (3). PR-mediated signaling is also important in the development of mammary tumorigenesis as shown by a markedly reduction in DMBA-initiated mammary carcinogenesis in PR null mice (4). Progesterone and estrogen alone or in combination enhances mammary tumorigenesis in p53-null mice (5), and treatment of Balb/c, but not C57BL/6 mice with medroxyprogesterone induces invasive ER+/PR+ ductal mammary carcinomas (6). In ovariectomized ACI rats, both estrogen and progesterone induce a 100% incidence of

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mammary tumors over 5-9 months (7). More importantly, PR regulation is also relevant to human breast cancer (8). PR and not ER is a marker for early stage breast cancer (9), and PR expression increases in tumors with BRCA1 mutations (10). Progestins, but not estrogens reactivate a subset of ER⁻/PR⁻/CK5⁺ cells with stem-like properties (11) that have the capacity to generate ER⁺/PR⁺/CK5⁻/CK18⁺ cells (12). This feature may be especially important with regard to the association between estrogen/progestin hormone replacement therapy (HRT) and increased risk of invasive lobular breast disease (13, 14), where the use of progestin oral contraceptives (15) and HRT (16) pose a greater breast cancer risk than estrogen alone.

It was appreciated early in the development of the PR antagonist, mifepristone/RU486, that it could serve as a contraceptive by interruption of the progesterone-dependent luteal phase of the menstrual cycle (17). Mifepristone, and second generation PR antagonists with greater PR selectivity, such as onapristone, have produced a high percentage of stable disease in postmenopausal women with metastatic breast cancer (18). Experimental studies have demonstrated that mifepristone can mitigate relapse to tamoxifen in MCF-7 breast cancer xenografts (19), inhibit the growth of progestin-stimulated T47-D and BT-474 xenografts (20) and prevented tumorigenesis resulting from inactivation of BRCA1 and p53 (21). In primary mammary tumor models, mifepristone inhibited DMBA- and NMU-induced tumorigenesis in rats (22, 23), which in one instance was not associated with classical PR-mediated effects (22). Importantly, mifepristone has high affinity for both the PR and glucocorticoid receptor (GR) (24), which may contribute to its antitumor activity in androgen-dependent and -independent prostate cancer (25).

In the present study, we examined whether mifepristone was equally efficacious as a chemopreventive agent in reducing DMBA mammary carcinogenesis in wild-type (WT) and MMTV-Pax8PPAR γ (Pax8) transgenic mice. The latter mouse model is unique in that the mammary gland expresses an increased percentage of ER⁺/PR⁺/CK5⁺/CK19⁺ and double CK14⁺/CK18⁺ progenitor cells, and presents with ER⁺/PR⁺ mammary carcinomas following progestin/DMBA-mediated carcinogenesis, in contrast to ER⁻/PR⁻ tumors of mixed lineage in wild-type mice (26). Thus, it was hypothesized that if mifepristone acted by inhibiting PR function, tumor formation in Pax8 mice would be more sensitive to mifepristone in comparison to wild-type mice. We report that in contrast to Pax8 animals, WT mice were more sensitive to a low dose of mifepristone, and that their response correlated with a unique gene expression signature. These results suggest that mifepristone acts through other signaling pathways apart from PR, and that further testing of mifepristone and other PR antagonists may be warranted in patients with breast cancer and other malignancies.

Materials and Methods

Mice

Pax8 transgenic FVB mice were generated as previously described (26). Age-matched WT FVB mice were obtained from Taconic Farms, Germantown, N.Y. All animal studies were conducted under protocols approved by the Georgetown University Animal Care and Use Committee.

Mammary carcinogenesis

Five week-old WT and Pax8 mice were treated with medroxyprogesterone acetate and DMBA as previously described (27, 28). Briefly, mice were injected s.c. with a single dose of 15 mg medroxyprogesterone acetate suspension (Depo-ProveraTM), and after seven days were administered four weekly doses of 1 mg DMBA/0.1 ml cottonseed oil by gavage.

Mifepristone was provided by the Chemoprevention Branch, NCI, and formulated as 2.5 mg and 7.5 mg 60-day controlled-release pellets and similarly formulated placebos by Innovative Research of America, Inc., Sarasota, FL. Pellets were implanted by trocar s.c. into the scapular region one day after the last dose of DMBA, and again after 60 days. The doses of mifepristone used in this study did not produce toxicity.

Antibodies

The source of antibodies, their dilution and use were the following: rabbit anti-ER α (sc-542, Santa Cruz Biotechnology, 1:200 for IHC, 1:1,000 for western); rabbit anti-PgR (sc-538, Santa Cruz Biotechnology, 1:200 for IHC, 1:1,000 for western); rabbit anti-GR α (sc-1004, Santa Cruz Biotechnology, 1:200 for IHC, 1:1,000 for western); rabbit anti-DKK2 (06-1087, Millipore Corp., 1:200, IHC); rabbit anti-Neu/ErbB2 (sc-33684, Santa Cruz Biotechnology, 1:200, IHC).

Immunohistochemistry

IHC analysis was carried out as previously described (26-28).

Western blotting

Western blotting was carried out as previously described (26). Briefly, tissue was frozen in liquid nitrogen and pulverized in a mortar and pestle, and mixed with lysis buffer containing: 0.1% SDS, 0.5% NP-40, phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 50 mM sodium fluoride, 10 mM β -glycerophosphate, 5 mM sodium pyrophosphate, and protease inhibitor cocktail (Roche Diagnostics). Following incubation on ice for 30 min, lysates were cleared by centrifugation for 15 min at $13,000 \times g$ at 4°C. Protein concentrations were determined by the Coomassie Plus Protein Assay (Pierce), and 50 μ g of lysate was separated in a 4-12% NuPAGE Bis-Tris gel (Invitrogen). After wet transfer, membranes were blocked for 1 hr at room temperature in TBS (pH 7.4) containing 5% non-fat dry milk and 0.1% Tween 20. Primary antibody was incubated overnight at 4°C, and secondary antibody was incubated for 1 hr at room temperature. Proteins were visualized with either SuperSignal West Pico or SuperSignal West Dura (Pierce).

Gene microarray analysis

RNA was obtained from 2-3 individual per group when animals were 5-6 months of age; only adenocarcinomas were analyzed and not tumors of other or mixed lineage. Total RNA was extracted using an RNeasy Mini Kit (Qiagen) following the manufacturer's protocol as previously described (26, 29), and the quality of RNA was confirmed by 28S/18S rRNA profiling using a microfluidic chip (Agilent). Equal amounts of RNA from each group were pooled for Affymetrix GeneChip® analysis. cRNA was synthesized using the Affymetrix (Santa Clara, CA) protocol with minor modifications as described (27). Biotin-labeled cRNA was fragmented for 35 min at 94°C and hybridized overnight to an Affymetrix mouse 430A 2.0 GeneChip® representing approximately 22,000 annotated mouse genes by the Macromolecular Analysis Shared Resource, Lombardi Comprehensive Cancer Center, Georgetown University. Hybridization signals were detected with an Agilent Gene Array scanner, and grid alignment and raw data generation performed with Affymetrix GeneChip® Operating software 1.1. Common genes expressed in tumors from WT and Pax8 mice treated with 2.5 mg mifepristone with a signal 300 ($\log_2$ 8.1) and 3-fold change (28-30) were analyzed by Pathway Studio 7.1 (Ariadne). Array data were deposited in the GEO database under accession no. GSE33753.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from 2-3 individual adenocarcinomas per group using an RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol as previously described (26, 29). The quality of RNA was confirmed by 28S/18S rRNA profiling using a microfluidic chip device, and equal amounts of RNA pooled from these samples for Affymetrix GeneChip analysis. Animals were 5-6 months of age when tumors were collected. One μg of RNA was reverse transcribed in a total volume of 20 μl using the Cloned AMV First-Strand cDNA Synthesis kit (Invitrogen). PCR was performed in triplicate in an ABI 7900 instrument (Applied Biosystems, Foster City, CA) using SYBRGreen detection (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. qRT-PCR primers were designed using the primer design tool at (31). Efficiencies of all primer sets (Table S1) were validated using a standard curve of five serial cDNA dilutions in water in duplicate. Primers were acceptable if the deviation from the slope of the standard curve was <0.3 , and if the melting curve showed only one product. The expression of each target gene was normalized to the expression of GAPDH, and the relative quantification method was applied using SDS2.3 software (Applied Biosystems, Foster City, CA). Primers are listed in Table S1.

Statistical analysis

Survival curves were analyzed by the Chi-square's log rank test and tumor incidence by the two-tailed paired Student's *t* test at a significance level *P* 0.05.

Results

The chemopreventive effect of RU486 is phenotype-selective

To evaluate the chemopreventive effect of mifepristone on breast cancer development, mammary carcinogenesis was induced by progesterin/DMBA in WT and Pax8 transgenic mice, and animals were administered two doses of either placebo, 2.5 mg or 7.5 mg mifepristone formulated as 60-day extended-release pellets 60 days apart beginning one day after the last dose of DMBA (Fig. 1). Survival was increased in WT mice treated with either 2.5 mg or 7.5 mg mifepristone (Fig. 1A), and correlated with reduced tumor formation (Fig. 1B). In contrast, Pax8 were responsive only to the higher dose of mifepristone (Fig. 1C, D). Assessment of the proliferative marker Ki-67 indicated somewhat higher expression in Pax8 vs. WT mice, and a greater reduction in WT vs. Pax8 mice following treatment with 2.5 mg mifepristone (Fig. S1A). Consistent with these results was a greater degree of nucleosome cleavage as assessed by TUNEL assay in mifepristone-treated WT mice vs. Pax8 mice (Fig. S1B). Dissipation of the effects of mifepristone after 120 days (Fig. 1) resulted in an increase in tumorigenesis in both WT and Pax8 mice, suggesting that mifepristone must be continuously present to produce an antitumor effect.

Gene expression analysis

Common genes differentially expressed in tumors from WT and Pax8 animals treated with either placebo or 2.5 mg mifepristone are presented in Table 1 and Fig. 2A,B. A complete list of 3-fold changes in gene expression is presented in Supplement Tables S2 and S3. The response of WT and Pax8 mice treated with 2.5 mg mifepristone was defined by a subset of 79 genes common to both groups. Of this subset, 52 genes responded in an opposite fashion (highlighted in **bold**), and distinguished the antitumor response in WT mice to 2.5 mg mifepristone from the lack of response of Pax8 mice at this dose. Pathway linkage analysis of these changes in gene expression is depicted in Fig. 3. Most notable was the association between an anti-inflammatory and anti-invasive gene signature in tumors from WT mice treated with mifepristone, as reflected in the marked reduction in expression of *Mmp13*,

Klk6, Klk7, S100a14, Saa1 and Saa2 (Table 1) and the gene ontology of these responses (Table 2). Interestingly, approximately 25% of the 52 genes are known to be regulated by PR and/or GR (Table 3), despite the lack of consistent changes in PR and GR expression by western blotting (Fig. S1C) and IHC (Fig. S1D). Additionally, there was no association between PR/GR-regulated genes and those regulated by ER (Table 3). Since expression of the Pax8PPAR γ transgene is under the control of the MMTV long terminal repeat, which is regulated by the GR (32), the expression of Pax8PPAR γ mRNA was assessed (Fig. 2C). There was no significant change in Pax8PPAR γ mRNA levels by mifepristone treatment. Another notable change specifically associated with the antitumor response of WT mice to 2.5 mg mifepristone was the 11-fold increase in Dkk2 mRNA (Fig. 2A) and protein (Fig. S1E), suggesting the possible involvement of Wnt pathway inhibition in the antitumor response to mifepristone.

Discussion

The aim of the present study was to assess the chemopreventive efficacy of mifepristone in a progestin-dependent mammary carcinogenesis model and to correlate therapeutic response with the gene expression profile of tumors induced in WT and Pax8 mice. Pax8 mice were previously found to express a stem/progenitor cell phenotype in the mammary gland, and to form ER⁺ adenocarcinomas exhibiting activation of Ras/ERK and ER signaling following progestin/DMBA-induced carcinogenesis (26). Interestingly, several genes previously identified with an ER⁺ cluster in human breast cancer (33) were preferentially expressed in Pax8 mice, viz. Fgfr2, Gata3, Slc39a8 and Stc2 (Table S3, Table S4). Pax8 mice also expressed Mmp11 identified in tumors with myoepithelial characteristics (34). Apart from these associations, it is difficult to draw further inferences about Pax8 mice with respect to a specific subtype of breast cancer. Nevertheless, Pax8 mice were more resistant to the lower dose of mifepristone than WT mice, whereas, the higher dose led to marked suppression of tumorigenesis in both groups of animals. It was previously reported that resistance to mifepristone in a progestin-dependent mouse mammary tumor correlated with high activation of ERK (35), a finding consistent with resistance in Pax8 mice to the lower dose of mifepristone. Mifepristone is known to block activation of IGF-1 signaling in MCF-7 breast cancer cells (36), and inhibition of this and other growth factor-dependent pathways may have also contributed to its chemopreventive effect. In this context, expression of the Wnt pathway inhibitor, Dkk2 (37), was markedly elevated in WT tumors sensitive to the lower dose of mifepristone (Fig. 2A, Fig. S1E, Table S2). This finding is consistent with the induction of Wnt4 expression in the mammary gland by progesterone and its role in ductal branching (38). In the endometrium, the progesterone-dependent luteal phase is associated with increased Wnt and reduced Dkk2 gene expression (39, 40), and is in agreement with similar changes in mammary tumors from mifepristone-sensitive WT mice.

One of the most notable features of mifepristone sensitivity in WT mice was the marked reduction in expression of genes associated with invasion and inflammation, including Klk6, Klk7, Mmp13, Saa1 and Saa2 (Table 1, Fig. 2B). Kallikreins are secreted proteases involved in extracellular matrix degradation and metastasis, and represent negative prognosticators for several malignancies, including breast cancer (41, 42). Not only is Klk6 a PR target gene (43), but it and other kallikrein members are involved in the regulation and activation of MMPs (41), including Mmp12 and Mmp13. The latter MMPs are associated with metastatic breast cancer (44), and Mmp13 has been linked to several inflammatory conditions and malignancies (45). In association with the reduction in invasive gene expression by mifepristone in WT tumors was the marked decrease in Saa1 and Saa2 expression. The serum amyloid A family are acute phase proteins that induce several MMPs (46) and are prognostic for reduced survival of breast cancer patients (47). Thus, a previously unrecognized feature of mifepristone appears to be its ability to suppress the expression of

several inflammatory and metastatic genes, and it will be of interest to assess whether it has anti-metastatic activity in an appropriate animal model or patient setting.

Mifepristone may also act through non-PR-dependent pathways, such as those related to GR and ERK signaling. In the context of our progestin-dependent mammary carcinogenesis model (27, 48), the involvement of GR and ERK are less unlikely. This conclusion is based, in part, on the lack of an effect by mifepristone on Pax8PPAR γ mRNA expression (Fig. 2C), and the regulation of the MMTV long terminal repeat by the GR (32, 49). In addition, mifepristone has been reported to inhibit ERK activation (50), which is associated with mammary tumors derived from MMTV-Pax8PPAR γ mice (26), which were resistant to the lower dose of mifepristone (Fig. 1). Overall, these results suggest that anti-GR activity is not a significant factor in the antitumor effects of low dose mifepristone treatment. To obviate the potential side effects of PR antagonists used for contraception, selective PR modulators (SPRMs) were developed with little or no antagonism of other receptors, such as GR by mifepristone (51). However, in contrast to the higher potency of SPRMs in uterine tissue, little change in potency or efficacy were observed for PR-selective agents, such as ORG31710, in comparison to mifepristone in DMBA-induced breast tumors in rats (18, 52). This suggests that the antitumor effects of PR antagonists may be dissociated from its anti-progestational effects (22, 53), and is consistent with the lesser sensitivity of Pax8 mice with greater levels of PR (Fig. 2C, Fig. S1D), in comparison to WT mice.

Previous clinical trials of mifepristone in relapsed postmenopausal breast cancer patients with PR+ metastatic disease who had received prior adjuvant hormone/chemotherapy produced only a partial response in approximately 10 percent of patients (54). The use of mifepristone as a second line intervention in patients previously treated with tamoxifen (55) or presenting with bone metastases (56) produced a similar outcome. Nevertheless, mifepristone exhibited synergistic inhibitory activity with tamoxifen and an aromatase inhibitor on mammary tumorigenesis (18), and similar effects were noted on breast cancer cells in culture (57, 58). The possible utility of PR antagonists is further suggested by the efficacy of mifepristone in an experimental model of Brca1-mediated tumorigenesis, where increased survival was attained (21). Thus, SPRMs have the potential to reduce the emergence of resistance to antihormone monotherapy and treat cancers highly resistant to most therapeutic modalities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Mifepristone inhibits mammary carcinogenesis in wild-type and MMTV-Pax8PPAR γ transgenic mice. (A) Survival curve in wild-type (*WT*) mice treated with mifepristone (*RU*). Each animal was injected s.c. with either a placebo or a 60-day controlled-release pellet of 2.5 or 7.5 mg mifepristone. Both doses of mifepristone produced a significant reduction in tumorigenesis (2.5 mg, $P=0.0438$; 7.5 mg, $P=0.0002$, by the Chi-square test). The effect produced by 2.5 mg mifepristone differed significantly from 7.5 mg mifepristone ($P=0.0268$). The number (*N*) of mice per group was: placebo ($N=10$), 2.5 mg mifepristone ($N=10$), 7.5 mg mifepristone ($N=9$). (B) Tumor formation in wild-type mice indicated in (A). Both doses of mifepristone produced a significant reduction in tumor formation (2.5 mg, $P=0.0640$; 7.5 mg, $P=0.0120$ by Student's two-tailed *t* test) vs. placebo-treated mice. (C) Survival curve in MMTV-Pax8PPAR γ (*Pax8*) transgenic mice treated with

mifepristone. Each animal was treated as in A). Only the 7.5 mg dose of mifepristone produced a significant reduction in tumorigenesis ($P=0.0001$ by the Chi-square test). The number (N) of mice per group was: placebo (N=9), 2.5 mg mifepristone (N=9), 7.5 mg mifepristone (N=7). (D) Tumor formation in MMTV-Pax8PPAR γ mice indicated in (C). Only the 7.5 mg dose of mifepristone produced a significant reduction in tumor formation ($P=0.0174$ by Student's two-tailed t test) vs. placebo-treated mice.

Fig. 2. qRT-PCR analysis of gene expression in tumors after mifepristone (*RU*) treatment of wild-type (*WT*) and MMTV-Pax8PPAR γ (*Pax8*) mice. (A) Representative changes in gene expression (Table S1 and Table S2) in tumors from WT and Pax8 mice. (B) Representative changes in gene (Table 1) that were opposite in WT and Pax8 mice. (C) MMTV-Pax8PPAR γ gene expression in tumors from Pax8 mice after treatment with 2.5 mg mifepristone as in Fig. 1.

Fig. 3. Signaling pathways associated with gene expression in tumors from wild-type mice responsive to mifepristone. Shown are genes common to both wild-type and MMTV-Pax8PPAR γ mice in response to 2.5 mg mifepristone as in Fig. 1. Genes highlighted in *blue* are those that changed in opposite directions in wild-type and transgenic mice.

Table 1
Gene expression in tumors common to wild-type and MMTV-Pax8PPAR γ mice treated with 2.5 mg mifepristone
Symbols in **bold** denote opposite responses between wild-type (*WT*) and MMTV-Pax8PPAR γ (*Pax8*) mice.

Symbol	Name	Log2 value		Ratio		Log2 value		Ratio	
		WT-Placebo	WT-RU	RU/Placebo	Pax8-Placebo	Pax8-RU	RU/Placebo		
Abcg2	ATP-binding cassette, sub-family G (WHITE), member 2	9.9	7.7	-4.8	8.8	6.5	-4.9		
Acs1l	acyl-CoA synthetase long-chain family member 1	10.5	7.8	-6.5	10.7	8.3	-5.5		
Acss1	acyl-CoA synthetase short-chain family member 1	9.4	7.2	-4.6	3.5	4.6	2.1		
Adipoq	adiponectin, C1Q and collagen domain containing	8.1	6.7	-2.6	11.8	6.0	-56.4		
Anxa8	annexin AB	9.8	7.2	-6.2	6.2	9.8	12.7		
Aqp1	aquaporin 1	8.6	10.6	4.0	9.5	7.9	-3.1		
Aqp3	aquaporin 3	9.2	5.4	-13.3	5.5	10.2	27.4		
Areg	amphiregulin	10.0	8.5	-2.7	11.0	8.2	-7.1		
Calca	calcitonin/calcitonin-related polypeptide, alpha	9.1	4.8	-19.9	9.9	3.5	-115.6		
Cdo1	cysteine dioxygenase 1, cytosolic	10.4	7.8	-6.1	12.3	8.8	-11.1		
Chpt1	choline phosphotransferase 1	9.6	7.9	-3.3	9.3	6.4	-8.2		
Ckmt1	creatine kinase, mitochondrial 1, ubiquitous	9.6	5.7	-14.8	8.5	10.2	3.1		
Cldn6	claudin 6	8.9	5.0	-15.0	10.7	9.1	-3.0		
Clec10a	C-type lectin domain family 10, member A	7.9	9.6	3.2	8.6	6.6	-4.2		
Col9a1	collagen, type IX, alpha 1	8.5	6.2	-5.0	8.9	7.0	-4.0		
Coro1a	coronin, actin binding protein 1A	9.9	11.6	3.1	10.8	9.1	-3.1		
Cretl	cysteine-rich C-terminal 1	8.5	6.3	-4.7	10.8	9.1	-3.1		
Cxd1	chemokine (C-X-C motif) ligand 1	8.2	6.5	-3.1	5.5	7.8	4.9		
Cxd5	chemokine (C-X-C motif) ligand 5	8.2	4.6	-12.3	2.9	5.0	4.5		
Cxd13	chemokine (C-X-C motif) ligand 13	4.6	9.1	22.1	9.6	4.8	-27.1		
Defb1	defensin beta 1	8.9	4.6	-16.6	11.1	7.7	-10.9		
Dgat2	diacylglycerol O-acyltransferase 2	7.3	6.3	-2.1	9.7	6.3	-10.2		
ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	10.3	6.6	-12.7	8.5	7.4	-2.1		
Fabp4	fatty acid binding protein 4, adipocyte	11.6	10.5	-2.2	11.5	8.7	-7.8		
Foxa1	forkhead box A1	7.4	5.1	-4.9	10.0	4.6	-41.8		
Fyb	FYN binding protein	8.0	9.6	3.1	8.7	6.5	-4.5		

Symbol	Name	Log2 value		Ratio		Log2 value		Ratio	
		WT-Placebo	WT-RU	RU/Placebo	Pax8-Placebo	Pax8-Placebo	Pax8-RU	RU/Placebo	Ratio
Gabrp	gamma-aminobutyric acid (GABA) A receptor, pi	8.7	6.8	-3.8	8.8	10.6	10.6	3.5	
Glycam 1	glycosylation dependent cell adhesion molecule 1	13.2	7.5	-51.7	11.6	8.7	8.7	-7.4	
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	9.1	6.9	-4.8	10.7	7.7	7.7	-7.6	
Gsta4	glutathione S-transferase, alpha 4	10.7	7.1	-12.5	10.4	12.6	12.6	4.5	
Igh-6	immunoglobulin heavy chain 6 (heavy chain of IgM)	6.1	9.4	10.6	9.3	6.3	6.3	-10.3	
Igk	immunoglobulin kappa chain complex	8.2	10.7	5.7	12.4	9.1	9.1	-10.5	
Il1r2	interleukin 1 receptor, type II	8.2	5.3	-7.6	5.2	7.0	7.0	3.6	
Irf8	interferon regulatory factor 8	7.2	8.9	3.3	8.4	6.8	6.8	-3.0	
Klk6	kallikrein related-peptidase 6	11.3	5.6	-50.3	4.9	8.6	8.6	12.6	
Klk7	kallikrein related-peptidase 7 (chymotryptic, stratum corneum)	9.6	4.7	-29.9	4.9	8.8	8.8	14.6	
Krt10	keratin 10	10.2	6.9	-9.6	8.2	9.7	9.7	3.0	
Krt14	keratin 14	12.0	7.4	-25.4	10.0	12.4	12.4	5.2	
Krt17	keratin 17	9.1	5.5	-12.2	8.4	10.9	10.9	6.0	
Krt19	keratin 19	12.1	9.4	-6.6	13.4	11.1	11.1	-5.1	
Krt23	keratin 23	9.2	5.8	-10.5	9.4	11.5	11.5	4.4	
Krt5	keratin 5	11.6	8.5	-8.0	10.8	12.6	12.6	3.5	
Krt79	keratin 79	8.6	4.9	-12.7	6.4	11.3	11.3	28.5	
Krt1ap	keratinocyte differentiation associated protein	9.2	4.9	-20.1	7.1	10.6	10.6	11.1	
Lalba	lactalbumin, alpha	10.4	4.7	-54.7	5.2	6.4	6.4	2.3	
Lao1	L-amino acid oxidase 1	11.7	3.8	-232.9	4.1	5.8	5.8	3.1	
Lpl	lipoprotein lipase	10.7	8.5	-4.6	12.0	8.3	8.3	-12.3	
Ltf	lactotransferrin	12.2	8.3	-15.2	9.1	11.3	11.3	4.9	
Ly6d	lymphocyte antigen 6 complex, locus D	7.5	5.1	-5.0	7.7	10.8	10.8	8.7	
Mmp10	matrix metalloproteinase 10	10.5	9.0	-2.7	3.8	9.8	9.8	64.5	
Mmp12	matrix metalloproteinase 12	9.3	6.5	-6.8	7.3	9.8	9.8	5.7	
Mmp13	matrix metalloproteinase 13	10.5	5.3	-35.6	3.1	9.7	9.7	94.8	
Moxd1	monooxygenase, DBH-like 1	10.6	8.3	-4.7	9.6	11.6	11.6	4.1	
Mtnr7	myotubularin related protein 7	8.0	5.9	-4.4	9.7	6.7	6.7	-8.1	
Muc15	mucin 15	11.3	5.9	-42.2	11.0	7.1	7.1	-15.3	
Myh11	myosin, heavy polypeptide 11, smooth muscle	10.2	7.7	-5.6	10.8	7.9	7.9	-7.2	

Symbol	Name	Log2 value		Ratio		Log2 value		Ratio	
		WT-Placebo	WT-RU	RU/Placebo	Pax8-Placebo	Pax8-RU	RU/Placebo		
Pck1	phosphoenolpyruvate carboxykinase 1, cytosolic	7.0	5.4	-3.0	10.2	3.8	-86.5		
Pcp4	Purkinje cell protein 4	9.0	6.4	-6.1	10.7	6.2	-22.4		
Plekhl1	pleckstrin homology domain containing, family B (evectins) member	8.7	6.3	-5.5	9.9	8.3	-3.1		
Plrlr	prolactin receptor	8.3	6.0	-5.0	10.1	5.9	-18.8		
Rorc	RAR-related orphan receptor gamma	8.8	5.9	-7.4	9.1	7.3	-3.6		
S100a14	S100 calcium binding protein A14	10.4	5.0	-39.9	8.9	11.3	5.4		
S100a8	S100 calcium binding protein A8 (calgranulin A)	9.4	7.3	-4.5	6.5	9.4	7.3		
S100a9	S100 calcium binding protein A9 (calgranulin B)	10.9	8.2	-6.3	8.6	11.2	5.9		
Saa1	serum amyloid A 1	12.1	4.2	-233.9	3.7	7.6	15.0		
Saa2	serum amyloid A 2	11.8	5.7	-70.0	5.1	7.9	7.3		
Saa3	serum amyloid A 3	11.8	7.4	-19.9	4.7	10.8	68.8		
Scd1	stearoyl-Coenzyme A desaturase 1	12.2	11.2	-2.0	12.9	10.6	-4.9		
Serpinb11	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member	8.7	2.9	-57.4	7.3	9.4	4.3		
Sh3bp5	SH3-domain binding protein 5 (BTK-associated)	9.7	7.5	-4.8	8.5	6.8	-3.3		
Sncg	synuclein, gamma	9.0	6.5	-5.9	8.5	6.6	-3.7		
Sprr1a	small proline-rich protein 1A	10.5	6.7	-14.2	8.8	11.9	8.9		
Stat5a	signal transducer and activator of transcription 5A	10.3	7.4	-7.1	9.5	7.8	-3.7		
Stfa3	stefin A3	8.7	3.7	-32.7	4.1	8.5	21.2		
Tchh	trichohyalin	8.9	4.4	-21.8	3.4	9.3	58.0		
Thrsp	thyroid hormone responsive SPOT14 homolog (Rattus)	11.7	7.0	-26.8	11.5	9.5	-4.1		
Trf	transferrin	12.9	11.3	-3.1	12.0	10.3	-3.2		
Vldlr	very low density lipoprotein receptor	8.4	6.6	-3.7	8.1	6.6	-3.0		
Zeb2	zinc finger E-box binding homeobox 2	8.9	10.8	3.8	9.3	7.9	-2.6		

Table 2

Ontology of gene expression negatively regulated in tumors from mifepristone-treated wild-type mice. Shown are the categories with enrichment data of $P < 0.05$ (Fisher's Exact test)

Name	Total Entities	Overlap	Overlapping Entities
epidermis development	102	7	SPRR1A,KRT10,KRT17,KRT14,KRT5,KLK7,KRTDAP
immune response	604	6	CXCL1,CXCL5,FYB,CXCL13,IRF8,CLEC10A
proteolysis	651	5	MMP12,MMP13,MMP10,KLK6,KLK7
metabolism	858	5	MMP12,MMP13,GSTA4,MMP10,ACSS1
chemotaxis	145	4	CXCL1,CXCL5,CXCL13,S100A8
cell-cell signaling	275	4	CXCL5,S100A9,CXCL13,LALBA
inflammation	293	4	CXCL1,S100A9,CXCL13,S100A8
transport	1807	4	AQP1,AQP3,LTF,GABRP
collagen catabolism	26	3	MMP13,MMP10,KLK6
glucose homeostasis	50	3	ADIPOQ,FOXA1,PCK1
drug response	295	3	MMP12,ERBB3,ADIPOQ
transcription	2246	3	IRF8,ZEB2,FOXA1
protein phosphorylation	739	2	ERBB3,FYB

Table 3

Genes with PR/GR and ER response elements in tumors common to wild-type and MMTV-Pax8PPAR γ mice treated with 2.5 mg mifepristone. Symbols in ***bold*** denote opposite responses as indicated in Table 1.

Symbol	Name	GRE/PRE	ERE
Acss1	acyl-CoA synthetase short-chain family member 1		+
Coro1a	coronin, actin binding protein 1A	+	
Defb1	defensin beta 1	+	
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)		+
Gsta4	glutathione S-transferase, alpha 4	+	
Krt10	keratin 10		
Krt14	keratin 14	+	
Krt17	keratin 17	+	
Krt23	keratin 23	+	
Krt5	keratin 5	+	
Krt79	keratin 79	+	
Mmp12	matrix metalloproteinase 12		+
Mmp13	matrix metalloproteinase 13	+	
Pck1	phosphoenolpyruvate carboxykinase 1, cytosolic	+	
prlr	prolactin receptor	+	
S100a14	S100 calcium binding protein A14	+	