[Frontiers in Bioscience 12, 3640-3647, May 1, 2007]

Progesterone-action in the murine uterus and mammary gland requires steroid receptor coactivator 2: relevance to the human

Rodrigo Fernandez-Valdivia[1], Atish Mukherjee[1], Paula Amato[2], D Craig. Allred[3], Jonathan Nguyen[1], Francesco J. DeMayo[1], John P. Lydon[1]

[1]Department of Molecular and Cellular Biology, [2]Department of Obstetrics and Gynecology, [3]Breast Center at Baylor College of Medicine and the Methodist Hospital, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030

TABLE OF CONTENTS

1. ABSTRACT

The importance of the progesterone receptor (PR) in female reproductive and mammary gland biology is well recognized; however, the coregulators selectively enlisted by PR have yet to be comprehensively defined *in vivo*. To evaluate the involvement of steroid receptor coactivator (SRC)/p160 family members in these physiological systems, a mouse model ($PR^{Cre/+}SRC\text{-}2^{flox/flox}$) was generated in which SRC-2 function was ablated specifically in cell-types that express the PR. Although $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ ovarian activity was normal, uterine function was severely compromised. Absence of SRC-2 in PR positive uterine cells led to an early block in embryo implantation, a defect not ascribed to SRC-1 or -3 knockouts. While the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uterus can display a partial decidual response, removal of SRC-1 in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uterus results in a block in decidualization, confirming that uterine SRC-2 and -1 are both necessary for PR-mediated transcriptional responses which lead to complete decidualization. The absence of significant branching and alveolar morphogenesis in the hormone-treated $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mammary gland establishes an important role for mammary SRC-2 in cellular proliferative programs that require PR. Finally, the observation that SRC-2 is also expressed in many of the same cell-types in the human, underscores the importance of further study of this coregulator's role in both peri-implantation biology and mammary development.

2. INTRODUCTION

2.1. Progesterone action in vivo: insights from the mouse

For over a decade, studies on the progesterone receptor knockout (PRKO) mouse have established PR as a critical coordinator of female reproductive biology (1). Removal of PR function not only undermined PRKO uterine function but severely compromised the ability of the hypothalamo-pituitary-ovarian axis to operate normally. Outside the female reproductive axis, the PRKO was instrumental in underscoring the importance of the P signal to mammary epithelial cell-division, a prerequisite for parity-induced mammary morphogenesis. Moreover, absence of mammary PR rendered the PRKO significantly less susceptible to mammary tumorigenesis (2-4), supporting a *bona fide* role for PR in both mammary morphogenesis and tumorigenesis. Importantly, these observations confirm a number of past experimental studies using the rodent model (5-8), human observational investigations (9-11), as well as recent clinical trials (12, 13).

Despite over a decade of PRKO studies, two important questions have yet to be comprehensively addressed: (1) what are the molecular pathways and networks which mediate the P signal to an appropriate physiological response in each target tissue? And (2) which coregulators (whether they be coactivators or corepressors)

are preferentially recruited by PR to modulate these downstream pathways? Although some advancement has been achieved to codify the transcriptome regulated by PR in some murine target tissues (for example, the uterus (14-20)), disclosing the key coregulators selectively utilized in PR mediated physiological responses is only now being achieved due in large part to the recent development of inventive genetic methods applied to the mouse.

2.2. The SRC/p160 family of coactivators

Past *in vitro* investigations revealed that PR transactivation is dependent on direct interactions with members of the steroid receptor coactivator (SRC/p160) gene family (21). The SRC family comprises three members: SRC-1 (ERAP140/ERAP160/NcoA-1), SRC-2 (TIF-2/GRIP-1/NcoA-2) and SRC-3 (p/CIP/RAC3/AIB1/TRAM-1/ACTR/NcoA-3); reviewed in (22). Sharing strong sequence homology, each of the three coactivators have been shown to directly bind the ligand binding domain of PR to enhance the transactivational potency of this nuclear receptor in the presence of ligand.

Knockout (KO) mouse models for each SRC member have recently been used to determine whether one or more SRC family members are required for physiological processes that depend on PR function. While the SRC-1KO survived to adulthood, its uterus was shown to undergo only a partial decidual response (23), supporting an important role for SRC-1 in uterine functional changes that require PR. Importantly, the partial decidual response of the SRC-1KO uterus implied additional coregulators are needed along with SRC-1 to achieve a full decidual response. Retarded ductal elongation and dichotomous branching in the mammary gland of the pubescent SRC-1KO female suggested SRC-1 may also be required for mammary morphogenetic effects that depend on estrogen (E) signaling (23).

Although a uterine defect was not detected in the SRC-3KO mouse (24), the SRC-3KO mammary gland exhibited a partial impairment in parity-induced ductal side-branching and alveologenesis, a mammary phenotype that parallels to some extent the PRKO mammary defect (1). Similar to the PRKO (2), the SRC-3KO mammary gland is significantly more resistant to mammary tumorigenesis (25) which indicates that mammary SRC-3 may be selectively recruited by a subgroup of PR-mediated transcriptional programs required for P-induced mammary morphogenesis and tumorigenesis. In aggregate, the SRC-KO studies suggest that SRC-1 has evolved as an important coactivator for uterine PR function, whereas SRC-3 is mandatory for a subset of mammary PR mediated events; investigations using the PR activity indicator (PRAI) model provide further support for this proposal (26) .

Unlike KOs for SRC-1 and -3, the SRC-2KO (referred to as the Transcriptional Intermediary Factor 2 KO or $TIF2^{-/-}$ from hereon) displays severe reproductive abnormalities in the both sexes (27). The $TIF2^{-/-}$ male is significantly subfertile with developmental defects in spermatogenesis. Initial study of the $TIF2^{-/-}$ female disclosed a marked hypofertility phenotype resulting from placental hypoplasia. Subsequent studies have revealed that $TIF2^{-/-}$ pups (male and female) are significantly underrepresented in litters from $TIF2^{+/-}$ intercrosses (unpublished observations); $TIF2^{-/-}$ females resulting from such crosses are infertile.

The overt reproductive phenotypes of the global KO for SRC-2—coupled with the recent observation that many of the murine cell-lineages that express PR also express SRC-2 (28)—suggested that SRC-2 may occupy a critical coactivator role in a subset of PR dependent physiological processes that are required for the maintenance of female fertility as well as for mammary morphogenesis and function. To address this proposal, an innovative $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic was created (28) by crossing the $PR^{Cre/+}$ knockin (29) with the $SRC\text{-}2^{flox/flox}$ model in which exon 11 encoding the nuclear receptor interacting domain (NID) is floxed to facilitate Cre-mediated excision (27). Because the resultant $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic is designed to postnatally ablate SRC-2 specifically in cell lineages that express the PR(28) , the embryonic, male reproductive, and the more recently reported metabolic (30) phenotypes exhibited by the $TIF2^{-/-}$ model are circumvented to enable evaluation of SRC-2's specific involvement in PR dependent transcriptional programs in the adult.

3. SRC-2 ABLATION IN MURINE PR POSITIVE CELL-LINEAGES

3.1. The $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ female exhibits an infertility defect

While female and male $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mice exhibited normal postnatal development, the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ female was shown to be infertile (28); in contrast male $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mice are fertile. Normal fertility exhibited by the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ male distinguishes this model from the $TIF2^{-/-}$ male which develops a severe hypofertility phenotype (27). The difference in phenotypes between the two KO models reflects the cell-lineage specific nature of the SRC-2 KO mutation in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ model. The absence of a metabolic phenotype in the both sexes of the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ model again reinforces the cell-selective manner in which SRC-2 is abrogated in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mouse; $TIF2^{-/-}$ mice have previously been shown to exhibit defects in energy homeostasis (30, 31).

Although SRC-2 is expressed in the murine ovary (28), an anovulatory phenotype was not observed in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ model. The absence of an ovulatory defect in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ model contrasts with the PRKO in which abrogation of intraovarian PR function results in a block in follicular rupture in the preovulatory ovary. The lack of an anovulatory phenotype to explain the infertility defect displayed by the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ model suggested that a different progestin target-site(s) in the reproductive axis must be compromised.

3.2. Removal of uterine SRC-2 results in embryo implantation failure and a partial decidual response

A failure to detect implantation sites in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uterus (5.5 days post-coitum (d.p.c.))

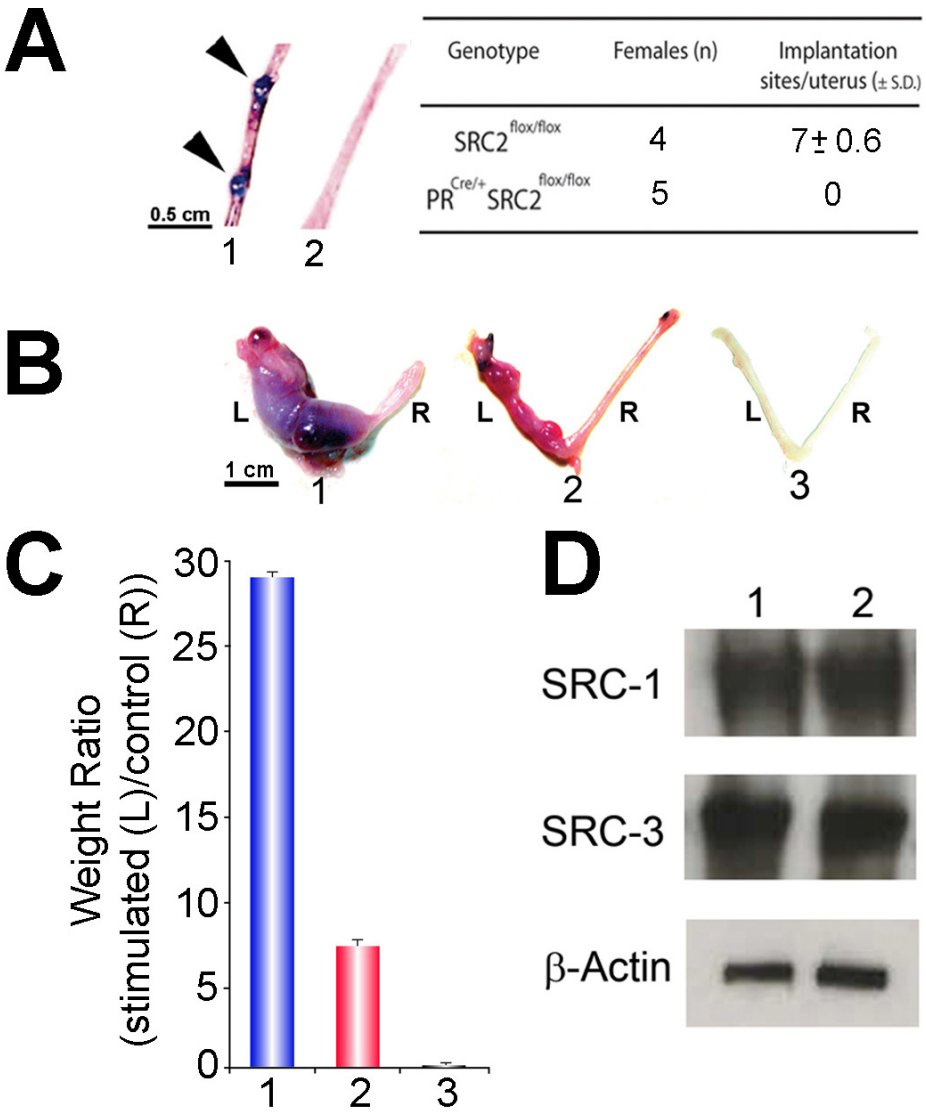

Figure 1. Embryo implantation and complete decidualization is blocked in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uterus. Panel A: Arrows show the location of implantation sites in the uterus (1) of a wild-type (WT) mouse (5.5 days post coitum (d.p.c.)). Implantation sites are revealed by the localized retention of Chicago blue dye (28). Importantly, implantation sites were not observed in similarly treated uteri taken from $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ (2) mice at 5.5 d.p.c. The average number of implantation sites per genotype per total number of mice analyzed is tabulated. Panel B displays the morphological response of the left (L) uterine horn to a deciduogenic stimulus for $SRC\text{-}2^{flox/flox}$ (1), $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ (2), and $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ SRC-1KO trigenic mice; for all genotypes, the right (R) uterine horn represents the unstimulated control. While the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uterus (2) exhibits a partial decidual response, notice the absence of a decidual response in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ SRC-1KO trigenic uterus (3). Panel C: The average weight ratios (± standard deviation (SD)) of stimulated (L) to control (R) horn for $SRC\text{-}2^{flox/flox}$, $PR^{Cre/+}SRC\text{-}2^{flox/flox}$, and $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ SRC-1KO trigenic uteri are shown. Panel D: Western analysis of uterine tissue derived from untreated adult virgin $SRC\text{-}2^{flox/flox}$ (1) and $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ (2) mice show no difference in expression levels for uterine SRC-1 and SRC-3 between the two genotypes (loading control is beta-actin). Modified from (28) (Copyright, 2006, Molecular and Cellular Biology).

not only accounted for the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ infertility defect but also for the fact that loss of uterine SRC-2 results in a block in the early progression of cellular processes in the receptive uterus that culminate in the establishment the maternofetal interface (Figure 1A). Moreover, while the $SRC\text{-}2^{flox/flox}$ uterus exhibits a full decidual response (Figure 1B and C), the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uterus displayed a partial decidual response to a deciduogenic stimulus (Figure 1B and C). This result supports the proposal that PR mediated transcription requires SRC-2 to launch a full uterine decidual response, and that other coregulators may be required in concert with SRC-2 to establish full decidualization. The partial decidual response exhibited by the SRC-1KO uterus (23) suggested that uterine SRC-2 and

SRC-1 are both required in PR-mediated transcriptional programs which establish full decidualization. To test this supposition, the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mouse was crossed with the SRC-1KO to create the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ SRC-1KO trigenic. Figure 1B and C clearly show that the uterus of the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ SRC-1KO trigenic is incapable of exhibiting any sign of a decidual reaction. The inability of the trigenic uterus to mount a decidual response establishes SRC-1 and SRC-2 as coactivators that are necessary and sufficient in launching a complete P-induced decidual response. Importantly, the expression levels of uterine SRC-1 and -3 were not changed in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ further underscoring the critical importance of uterine SRC-2 alone in the development of the receptive and decidualized uterus (Figure 1D).

3. 3. Progestin-dependent mammary morphogenesis requires SRC-2

The expression of SRC-2 in mammary PR positive cells (28) suggested that this coactivator may occupy a role in PR-mediated signaling that results in ductal side-branching and alveolar morphogenesis in the adult gland. This assumption was confirmed by the fact that the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mouse failed to exhibit mammary morphogenesis that is typical following a standard 3-week EP treatment regimen (Figure 2A-D). As previously described for the PRKO phenotype, the underlying cause of the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mammary defect was an inability of its luminal epithelium to undergo proliferation in response to hormone exposure (Figure 2E). These results establish an obligatory role for SRC-2 function in PR mediated transcriptional programs that are required for mammary ductal side-branching and alveologenesis, morphological changes that usually occur with the onset of pregnancy.

4. RELEVANCE TO THE HUMAN

As previously reported (32), the transactivational potency of human PR is markedly elevated with increasing levels of human SRC-2 (Figure 3A), supporting an important coactivator role for SRC-2 in progestin-dependent physiological responses in the human. Furthermore, immunohistochemistry reveals that SRC-2 is expressed in a subset of human steroid-responsive target tissues (Figure 3B-F). In human prostate (Figure 3B), SRC-2 expression is spatially confined to the epithelial compartment, a known target-site for androgen receptor-mediated signaling (33-35). Of relevance to this review, SRC-2 immunoreactivity is evident in a subgroup of epithelial cells in the normal human breast (Figure 3C and D); like the murine gland (28), SRC-2 is not expressed in the stroma. The heterogeneous spatial expression pattern for SRC-2 in the human mammary epithelium mirrors SRC-2's spatial expression profile in the murine gland (28) in which SRC-2 colocalizes with PR expression. As a corollary, PR has also been shown to display a punctate pattern of expression in the human breast (36), whether SRC-2 and PR colocalize in the human breast remains to be addressed. Examining this issue will be important as separation of PR positive mammary epithelial cells from proliferating cells is now accepted as a conserved cellular arrangement shared by rodent and human mammary tissue that supports a paracrine mode of action for mammary PR in the normal breast, reviewed in (37). Based on similar regional expression patterns for mammary SRC-2 and PR in the rodent and human in conjunction with the observation that SRC-2 ablation in the murine mammary gland results in a morphological defect akin to the PRKO suggests that SRC-2 may have an important role not only in PR's paracrine mode of action in the normal breast but also in an autocrine mechanism which has been proposed to explain aberrant steroid hormone signaling in a subset of rodent and human breast cancers, reviewed in (37).

The most conspicuous phenotype exhibited by the $PR^{cre/+}SRC\text{-}2^{flox/flox}$ female is an infertility defect, which results from an inability to manifest a receptive uterus for embryo implantation (Figure 1). Immunohistochemical investigations have shown that uterine SRC-2 and PR localize to identical cell-types in the murine uterus (28), supporting direct involvement of this coactivator in PR-mediated signaling pathways that are mandatory for uterine cellular changes that are required for embryo implantation and subsequent decidualization. Although it is not clear whether SRC-2 exhibits similar effects in the human uterus, recent immunohistochemical studies clearly show that SRC-2 and PR are expressed in many of the same cell-types of the human uterus (Figure 3G-F) that have been described for the mouse (28). From a clinical perspective, recurrent implantation failure is now accepted as a key underlying factor that negates the establishment of a successful pregnancy (either by natural means or by assisted reproductive technologies (ART) (38)). Although the information is currently sparse regarding the involvement of SRC-2 in the human endometrium, recent reports have described abnormal elevations in SRC-2 levels in endometrial biopsies from infertile women with polycystic ovarian syndrome (PCOS) as well as in a subset of endometrial cancers (39, 40), suggesting a possible role for this coactivator in human endometrial disorders. Because of the common use of progestins in the management of female reproductive health, coupled with our recent finding that SRC-2 is as an essential coactivator for PR action in female reproduction, we believe SRC-2 could represent a new molecular marker in diagnostic reproductive medicine and/or a future intervention target for the treatment of gynecologic pathologies.

5. PERSPECTIVE

This review describes how state-of-the-art murine genetic approaches uncovered a critical coactivator role for SRC-2 in PR-mediated signaling in both the uterus and mammary gland, with attendant implications for this coactivator's influence in similar systems in the human. Noticeably distinct from SRC-1 and SRC-3, whose coactivator properties are primarily dedicated toward transcriptional responses in the uterus and mammary gland respectively, SRC-2 is shown to be an indispensable PR coactivator in both target tissues. Because identification of tissue-specific coregulators that are preferentially recruited by PR is now recognized as a prerequisite to further understanding why different target tissues exhibit different

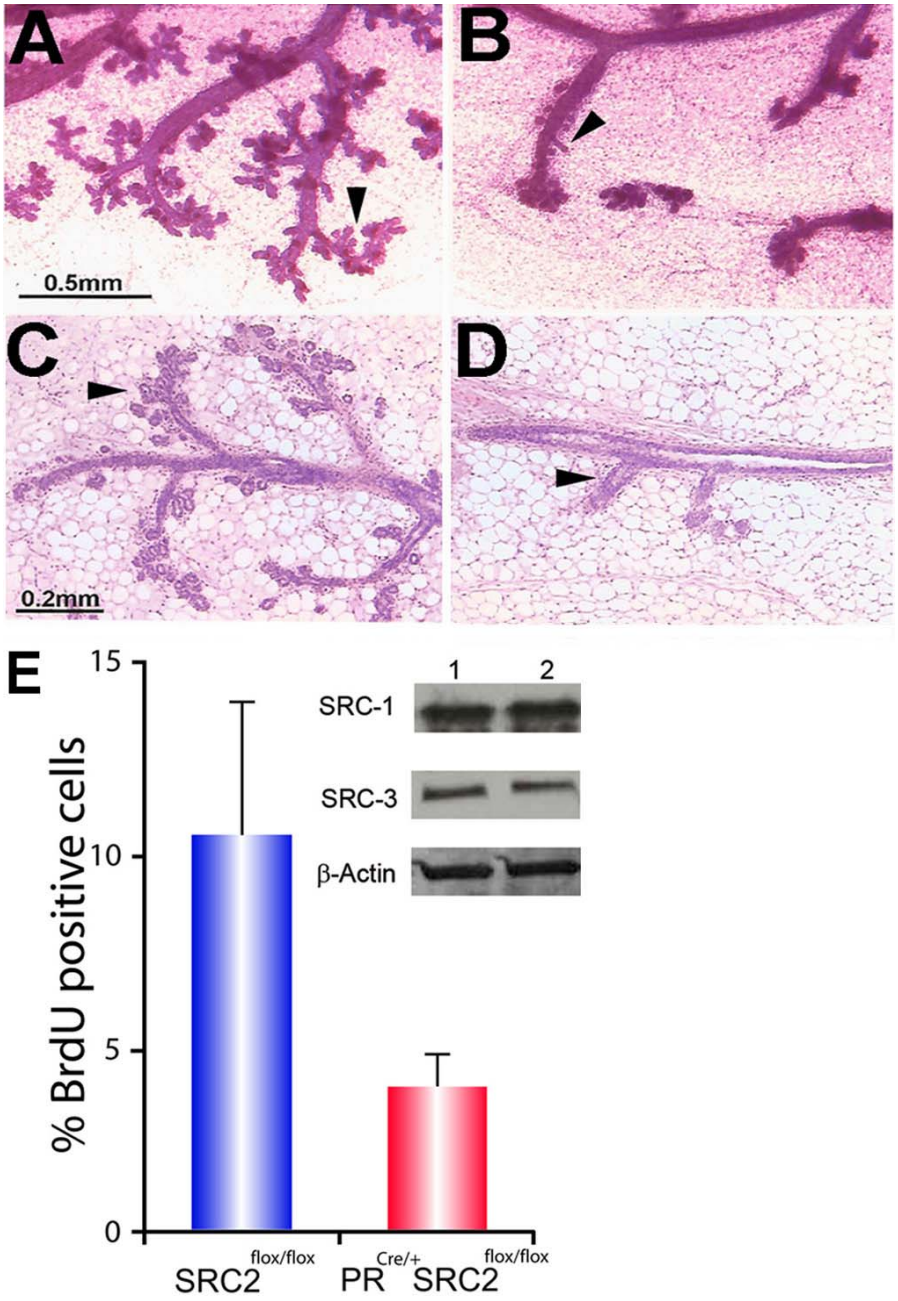

Figure 2. Branching morphogenesis is severely comprised in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mammary gland. Panels A and B show whole-mounts of inguinal mammary glands from EP-treated $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mice respectively. Compared to the EP-treated $SRC\text{-}2^{flox/flox}$ gland, note the significant reduction in ductal side-branching and alveologenesis (black arrow) in the EP-treated $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ gland. Panels C and D are hematoxylin and eosin (HE) stained sections of glands shown in panels A and B respectively; compared to the EP-treated $SRC\text{-}2^{flox/flox}$ gland, the marked decrease in epithelial content in the similarly treated $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ gland is indicated by the arrow. Panel E: The average percentage of mammary epithelial cells (± S.D.) scoring positive for BrdU staining in the EP-treated $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ glands is tabulated (BrdU incorporation indicates cells in S-phase of the cell-cycle). The inset displays a Western for mammary SRC-1 and -3 for $SRC\text{-}2^{flox/flox}$ (1) and $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ (2) mice. Changes in the levels of SRC-1 and -3 are not detected in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mammary gland (beta-actin is a loading control). Scale bars in panels A and C apply to B and D respectively. Modified from (28) (Copyright, 2006, Molecular and Cellular Biology).

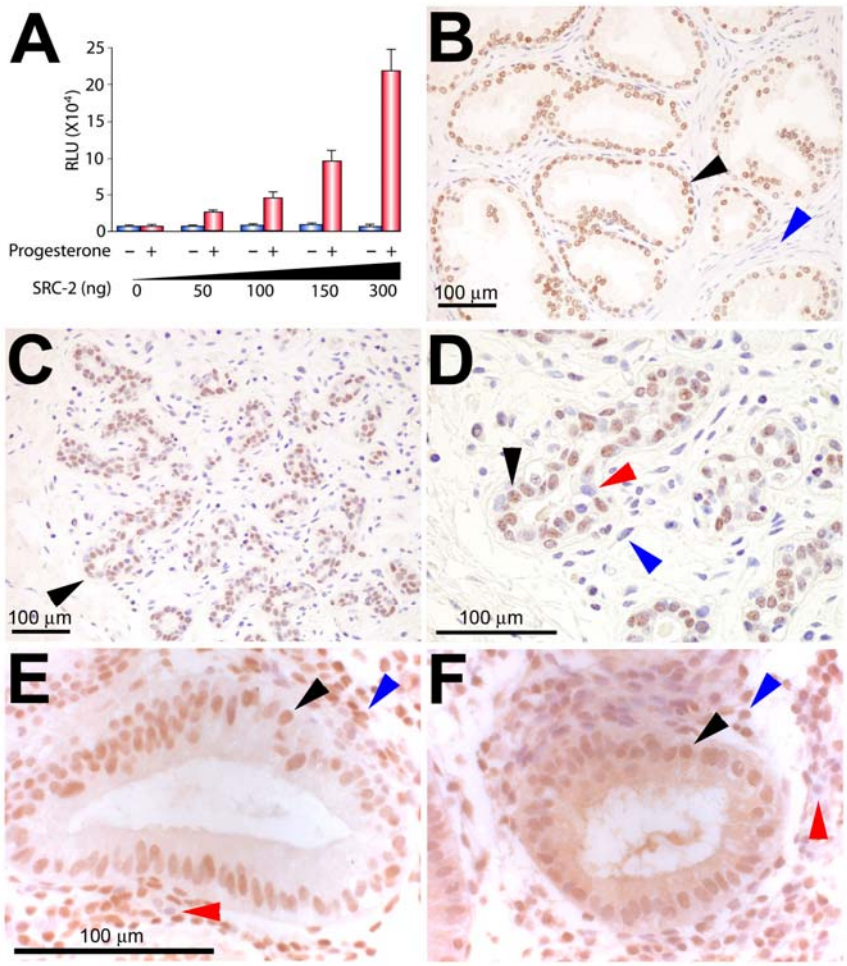

Figure 3. Human steroid hormone responsive tissues express SRC-2. Panel A shows that the increase in transactivational functionality of human PR-B is dependent on increased levels of human SRC-2 (red bars ± S.D.) in the presence of P; in the absence of ligand this increase is not observed (blue bars). As described previously (41), human PR-B; SRC-2 (both cloned into pCR3.1) and the luciferase reporter pGRE.E1b.LUC were transiently cotransfected into HeLa cells in the presence or absence of 10^{-7}M R5020; results are expressed in relative light units (RLU). Panel B shows SRC-2 is expressed in the majority of epithelial cells of the human prostate (black arrow), an established cellular target for androgen receptor action (33); note: the stromal compartment scores negative for SRC-2 expression (blue arrow). Panel C shows a representative example of a normal type 1 terminal ductal lobular unit (TDLU) of the human breast in which SRC-2 expression is contained within the epithelial compartment (black arrow). Panel D is a higher magnification of the region indicated by the black arrow in panel C. Note that SRC-2 expression is confined to a subset of epithelial cells of the TDLU (black arrow indicates an epithelial cell scoring positive for SRC-2 expression whereas the red arrow highlights an epithelial cell which is negative for SRC-2 expression; blue arrow denotes a stromal cell which is negative for SRC-2 expression). The spatial pattern of mammary SRC-2 expression resembles that previously reported for ER-alpha and PR expression in the human breast (36). Panels E and F show sections of the glandular epithelial compartment (with associated stroma) of the human endometrium stained for PR and SRC-2 expression respectively. Note that PR and SRC-2 are detected in nuclei of the same cell-types in the glandular epithelium and stromal compartment (black and blue arrows respectively). The red arrow in panels E and F highlights stromal cells that are negative for PR and SRC-2 expression respectively; scale bar in panel E applies to panel F. Endometrial biopsies were obtained by endometrial pipelle from normally cycling women, aged between 18-35 years, during the mid-secretory (luteal) phase of the menstrual cycle (days 20-24 which is based on the ideal 28 day cycle in which day 1 represents the first day of menstrual flow and day 14 the day of ovulation). Cycle phase was determined relative to the timing of the urinary luteinizing hormone (LH) surge. Human SRC-2 and PR immunohistochemical detection was performed by established methods previously reported by our group (28, 42). With institutional review board approval, human tissue samples were obtained from Baylor College of Medicine affiliated hospitals. Reproduced with permission from (43) (Copyright, 2006, Molecular and Cellular Biology).

responses to progestins, we believe that continued studies of SRC-2 action *in vivo* are warranted to further illuminate this understudied but important area of endocrine research.

6. ACKNOWLEDGEMENTS

We thank Drs Pierre Chambon and Martine Gehin, Institut Clinique de la Souris, (ICS-IGBMC), BP10142, 67404 ILLKIRCH Cedex France, for kindly providing the floxed SRC-2 mouse model and Dr. Jun Qin, Baylor College of Medicine, for the SRC-2 antibody. The technical assistance of Jie Li, Yan Ying, and Jie Han is gratefully acknowledged. This research was supported by NIH and private grants HD-42311 (F. J. D.), and CA-07730 and Susan G. Komen Breast Research Cancer Program (J. P.L.).

7. REFERENCES

1. J. P. Lydon, F. J. DeMayo, C. R. Funk, S. K. Mani, A. R. Hughes, C. A. Montgomery Jr., G. Shyamala, O. M. Conneely & B. W. O'Malley: Mice lacking progesterone receptors exhibit pleiotropic reproductive abnormalities. *Genes and Dev.*, 9, 2266-2278 (1995)
2. J. P. Lydon, G. Ge, F. S. Kittrell, D. Medina & B. W. O'Malley: Murine mammary gland carcinogenesis is critically dependent on progesterone receptor function. *Cancer Res*, 59, 4276-4284 (1999)
3. R. T. Chatterton, Jr., J. P. Lydon, R. G. Mehta, E. T. Mateo, A. Pletz & V. C. Jordan: Role of the progesterone receptor (PR) in susceptibility of mouse mammary gland to 7,12-dimethylbenz[a]anthracene-induced hormone-independent preneoplastic lesions in vitro. *Cancer Lett*, 188, 47-52 (2002)
4. D. Medina, F. S. Kittrell, A. Shepard, A. Contreras, J. M. Rosen & J. Lydon: Hormone dependence in premalignant mammary progression. *Cancer Res*, 63, 1067-72 (2003)
5. J. W. Jull: The effects of oestrogens and progesterone on the chemical induction of mammary cancer in mice of the *IF* strain. *J. Path. Bact.*, 68, 547-559 (1954)
6. S. P. Robinson, & V. C. Jordan: Reversal of antitumor effects of tamoxifen by progesterone in the 7, 12-dimethylbenzanthracene-induced rat mammary carcinoma model. *Cancer Res.*, 47, 5386-5390 (1987)
7. I. H. Russo, & J. Russo: Progestagens and mammary gland development : differentiation versus carcinogenesis. *Acta Endocrinol.*, 125, 7-12 (1991)
8. C. M. Aldaz, Q. Y. Liao, M. La Bate & D. A. Johnston: Medroxyprogesterone acetate accelerates the development and increases the incidence of mouse mammary tumors induced by dimethylbenzanthracene. *Carcinogenesis*, 17, 2069-2072 (1996)
9. G. A. Colditz, K. M. Egan & M. J. Stampfer: Hormone replacement therapy and risk of breast cancer: results from epidemiologic studies. *Am J Obstet Gynecol*, 168, 1473-80. (1993)
10. C. Schairer, J. Lubin, R. Troisi, S. Sturgeon, L. Brinton & R. Hoover: Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk [see comments]. *JAMA*, 283, 485-91 (2000)
11. R. K. Ross, A. Paganini-Hill, P. C. Wan & M. C. Pike: Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. *J Natl Cancer Inst*, 92, 328-32 (2000)
12. J. E. Rossouw, G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. L. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard, K. C. Johnson, J. M. Kotchen & J. Ockene: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*, 288, 321-33 (2002)
13. V. Beral,: Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet*, 362, 419-27 (2003)
14. S. K. Das, I. Chakraborty, B. C. Paria, X. N. Wang, G. Plowman & S. K. Dey: Amphiregulin is an implantation-specific and progesterone-regulated gene in the mouse uterus. *Mol Endocrinol*, 9, 691-705 (1995)
15. H. Lim, L. Ma, W. G. Ma, R. L. Maas & S. K. Dey: Hoxa-10 regulates uterine stromal cell responsiveness to progesterone during implantation and decidualization in the mouse. *Mol Endocrinol*, 13, 1005-17. (1999)
16. C. Brisken, A. Heineman, T. Chavarria, B. Elenbaas, J. Tan, S. K. Dey, J. A. McMahon, A. P. McMahon & R. A. Weinberg: Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. *Genes Dev*, 14, 650-4 (2000)
17. Y. P. Cheon, Q. Li, X. Xu, F. J. DeMayo, I. C. Bagchi & M. K. Bagchi: A Genomic Approach to Identify Novel Progesterone Receptor Regulated Pathways in the Uterus during Implantation. *Mol Endocrinol*, 16, 2853-71 (2002)
18. N. Takamoto, B. Zhao, S. Y. Tsai & F. J. DeMayo: Identification of Indian hedgehog as a progesterone-responsive gene in the murine uterus. *Mol Endocrinol*, 16, 2338-48 (2002)
19. P. M. Ismail, F. J. DeMayo, P. Amato & J. P. Lydon: Progesterone induction of calcitonin expression in the murine mammary gland. *J Endocrinol*, 180, 287-295 (2004)
20. J. W. Jeong, K. Y. Lee, I. Kwak, L. D. White, S. G. Hilsenbeck, J. P. Lydon & F. J. DeMayo: Identification of murine uterine genes regulated in a ligand-dependent manner by the progesterone receptor. *Endocrinology*, 146, 3490-505 (2005)
21. N. J. McKenna, & B. W. O'Malley: Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell*, 108, 465-74 (2002)
22. D. M. Lonard, & B. W. O'Malley: Expanding functional diversity of the coactivators. *Trends Biochem Sci*, 30, 126-32 (2005)
23. J. Xu , Y. Qiu, F. J. DeMayo, S. Y. Tsai, M. J. Tsai & B. W. O'Malley: Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. *Science*, 279, 1922-1925 (1998)
24. J. Xu, L. Liao, G. Ning, H. Yoshida-Komiya, C. Deng & B. W. O'Malley: The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. *Proc Natl Acad Sci U S A*, 97, 6379-84 (2000)
25. S. Q. Kuang, L. Liao, S. Wang, D. Medina, B. W. O'Malley & J. Xu: Mice lacking the amplified in breast cancer 1/steroid receptor coactivator-3 are resistant to

chemical carcinogen-induced mammary tumorigenesis. *Cancer Res*, 65, 7993-8002 (2005)
26. S. J. Han, F. J. DeMayo, J. Xu, S. Y. Tsai, M. J. Tsai & B. W. O'Malley: Steroid receptor coactivator (SRC)-1 and SRC-3 differentially modulate tissue-specific activation functions of the progesterone receptor. *Mol Endocrinol*, 20, 45-55 (2006)
27. M. Gehin, M. Mark, C. Dennefeld, A. Dierich, H. Gronemeyer & P. Chambon: The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and p/CIP. *Mol Cell Biol*, 22, 5923-37 (2002)
28. A. Mukherjee, S. M. Soyal, R. Fernandez-Valdivia, M. Gehin, P. Chambon, F. J. Demayo, J. P. Lydon & W. O'Malley B: Steroid receptor coactivator 2 is critical for progesterone-dependent uterine function and mammary morphogenesis in the mouse. *Mol Cell Biol*, 26, 6571-83 (2006)
29. S. M. Soyal, A. Mukherjee, K. Y. Lee, J. Li, H. Li, F. J. DeMayo & J. P. Lydon: Cre-mediated recombination in cell lineages that express the progesterone receptor. *Genesis*, 41, 58-66 (2005)
30. J. W. Jeong, I. Kwak, K. Y. Lee, L. D. White, X. P. Wang, F. C. Brunicardi, W. O'Malley B & F. J. Demayo: The Genomic Analysis of the Impact of Steroid Receptor Coactivators (SRCs) Ablation on Hepatic Metabolism. *Mol Endocrinol* (2006)
31. F. Picard, M. Gehin, J. Annicotte, S. Rocchi, M. F. Champy, B. W. O'Malley, P. Chambon & J. Auwerx: SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell*, 111, 931-41 (2002)
32. K. Hofman, J. V. Swinnen, G. Verhoeven & W. Heyns: Coactivation of an endogenous progesterone receptor by TIF2 in COS-7 cells. *Biochem Biophys Res Commun*, 295, 469-74 (2002)
33. Z. Culig, H. Klocker, G. Bartsch & A. Hobisch: Androgen receptors in prostate cancer. *Endocr Relat Cancer*, 9, 155-70 (2002)
34. C. A. Berrevoets, A. Umar, J. Trapman & A. O. Brinkmann: Differential modulation of androgen receptor transcriptional activity by the nuclear receptor co-repressor (N-CoR). *Biochem J*, 379, 731-8 (2004)
35. X. Ye, S. J. Han, S. Y. Tsai, F. J. DeMayo, J. Xu, M. J. Tsai & B. W. O'Malley: Roles of steroid receptor coactivator (SRC)-1 and transcriptional intermediary factor (TIF) 2 in androgen receptor activity in mice. *Proc Natl Acad Sci U S A*, 102, 9487-92 (2005)
36. R. B. Clarke, A. Howell, C. S. Potten & E. Anderson: Dissociation between steroid receptor expression and cell proliferation in the human breast. *Cancer Res*, 57, 4987-4991 (1997)
37. R. Fernandez-Valdivia, A. Mukherjee, B. Mulac-Jericevic, O. M. Conneely, F. J. DeMayo, P. Amato & J. P. Lydon: Revealing progesterone's role in uterine and mammary gland biology: insights from the mouse. *Semin Reprod Med*, 23, 22-37 (2005)
38. E. R. Norwitz, D. J. Schust & S. J. Fisher: Implantation and the survival of early pregnancy. *N Engl J Med*, 345, 1400-8 (2001)
39. C. W. Gregory, E. M. Wilson, K. B. Apparao, R. A. Lininger, W. R. Meyer, A. Kowalik, M. A. Fritz & B. A. Lessey: Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. *J Clin Endocrinol Metab*, 87, 2960-6 (2002)
40. N. Pathirage, L. A. Di Nezza, L. A. Salmonsen, T. Jobling, E. R. Simpson & C. D. Clyne: Expression of aromatase, estrogen receptors, and their coactivators in patients with endometrial cancer. *Fertil Steril*, 86, 469-72 (2006)
41. D. M. Lonard, S. Y. Tsai & B. W. O'Malley: Selective estrogen receptor modulators 4-hydroxytamoxifen and raloxifene impact the stability and function of SRC-1 and SRC-3 coactivator proteins. *Mol Cell Biol*, 24, 14-24 (2004)
42. S. Lee, S. K. Mohsin, S. Mao, S. G. Hilsenbeck, D. Medina & D. C. Allred: Hormones, receptors, and growth in hyperplastic enlarged lobular units: early potential precursors of breast cancer. *Breast Cancer Res*, 8, 1-9 (2005)
43. A. Mukherjee, P. Amato, D. C. Allred, R. Fernandez-Valdivia, J. Nguyen, W. O'Malley B, F. J. DeMayo & J. P. Lydon: Steroid receptor coactivator 2 is essential for progesterone-dependent uterine function and mammary morphogenesis: insights from the mouse--implications for the human. *J Steroid Biochem Mol Biol*, In press, (2006)

Abbreviations: PR: progesterone receptor, PRKO: progesterone receptor knockout, ER: estrogen receptor alpha, SRC-2: steroid receptor coactivator 2, TIF2: transcriptional intermediary factor 2, NID: nuclear interacting domain, ART: artificial reproductive technologies, PCOS: polycystic ovarian syndrome

Key Words: Progesterone, Steroid Receptor Coactivator-2, Mouse, Human, Uterus, Implantation, Decidualization, Mammary Gland, Branching Morphogenesis, Review

Send correspondence to: John P. Lydon, Ph.D., Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030, Tel: 713-798-3534, Fax: 713-790-1275, E-mail: jlydon@bcm.tmc.edu

http://www.bioscience.org/current/vol12.htm