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# Progesterone and Overlooked Endocrine Pathways in Breast Cancer Pathogenesis

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Worldwide, breast cancer incidence has been increasing for decades. Exposure to reproductive hormones, as occurs with recurrent menstrual cycles, affects breast cancer risk, and can promote disease progression. Exogenous hormones and endocrine disruptors have also been implicated in increasing breast cancer incidence. Numerous in vitro studies with hormone-receptor-positive cell lines have provided insights into the complexities of hormone receptor signaling at the molecular level; in vivo additional layers of complexity add on to this. The combined use of mouse genetics and tissue recombination techniques has made it possible to disentangle hormone action in vivo and revealed that estrogens, progesterone, and prolactin orchestrate distinct developmental stages of mammary gland development. The 2 ovarian steroids that fluctuate during menstrual cycles act on a subset of mammary epithelial cells, the hormone-receptor-positive sensor cells, which translate and amplify the incoming systemic signals into local, paracrine stimuli. Progesterone has emerged as a major regulator of cell proliferation and stem cell activation in the adult mammary gland. Two progesterone receptor targets, receptor activator of NfκB ligand and Wnt4, serve as downstream paracrine mediators of progesterone receptor-induced cell proliferation and stem cell activation, respectively. Some of the findings in the mouse have been validated in human *ex vivo* models and by next-generation whole-transcriptome sequencing on healthy donors staged for their menstrual cycles. The implications of these insights into the basic control mechanisms of mammary gland development for breast carcinogenesis and the possible role of endocrine disruptors, in particular bisphenol A in this context, will be discussed below. **(*Endocrinology* 156: 3442–3450, 2015)**

Breast cancer affects 1 in 8 women in Western countries (1). The disease is heterogeneous: more than 20 distinct histopathological subtypes are recognized (2). Of clinical relevance are tumor grade and tumor stage, as well as classification according to estrogen receptor (ER)α and progesterone receptor (PR) status, as assessed by immunohistochemistry (IHC), and HER2 overexpression due to amplification, as determined by IHC and fluorescent in situ hybridization. Five major molecular breast cancer subtypes were discerned by global gene expression profiling and largely correspond to IHC subtype, with luminal A representing ER+, of low grade and low Ki67 index; Luminal B, ER+ of higher grade and proliferative index; HER2 being HER2+ by IHC and either ER+ or ER−; and the "basal-like," which are dubbed triple negative because they do not express any of the 3 receptors. The last is a heterogeneous group that contains further subtypes (3). More than 2 thirds of all breast cancers are luminal, ie, ER+, and differ in biology and clinical course from HER2+ and basal-like tumors (4).

Tamoxifen is a selective ER modulator, which was introduced over 40 years ago and has dramatically increased survival of ER+ breast cancer patients (5). ER signaling can now also be inhibited by pure ER antagonists, such as fulvestrant, or indirectly by aromatase inhibitors, which are the mainstay in the therapy of most postmenopausal breast cancer patients. Although most ER+ tumors express ER in at least 90% of the cells, some cancers have

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Abbreviations: AR, androgen receptor; BPA, bisphenol A; DES, diethylstilbestrol; ER, estrogen receptor; HRT, hormone replacement therapy; IHC, immunohistochemistry; PR, progesterone receptor; RANKL, receptor activator of NfκB ligand; VDR, vitamin D receptor.

lower percentages of ER+ tumor cells, but are still classified as ER+ as long as at least 1% of the tumor cells express ER (6). The ER signaling pathway has long been a major focus of research in the breast cancer field; although it is of premier importance in the therapy of ER+ breast cancer, other hormonal factors are increasingly considered to play an important role in the pathogenesis of the disease (7–9). Here, we will discuss how endogenous hormones, in particular progesterone, impinge on the breast and their role in tumor development, and we will attempt to integrate exposure to endocrine disruptors into this picture, taking the widely distributed bisphenol A (BPA) as an example. New insights in this area are relevant to breast cancer prevention but may also be explored for novel therapeutic approaches.

## Hormonal risk factors

Ovariectomy (10) was shown to benefit individual breast cancer patients more than 100 years ago. Epidemiological studies revealed that breast cancer risk increases with the number of menstrual cycles a woman experiences in her lifetime (11): early menarche, late menopause, and short menstrual cycles all increase risk (12). Based on breast cancer statistics from the seventies that were not confounded by hormone replacement therapy (HRT), Pike et al (11) calculated that if it were not for menopause there would be 6 times as many cases of breast cancer (11, 13). More recently, it was shown that the risk related to menstrual cycles applies to all subtypes of breast cancer (14). Young age at first pregnancy has a protective effect (15, 16); more detailed data from the Nurses Health Study indicates that this applies to hormone-receptor-positive, more specifically PR+, breast cancers (14). The protective effects of early pregnancy rely on a number of factors: lower levels of GH (17) and prolactin (18) after a first pregnancy, changes in stem cell numbers and biology, changes in p53 functional status (19), and differences in the proliferative response have all been implicated (20).

Large women's health studies revealed that breast cancer risk related to HRT increases when an estrogenic compound is combined with progestin (21–23), whereas estrogens on their own can have protective effects (24). Indeed, since HRT was discontinued, breast cancer incidence has diminished (25). Similarly, women who are currently on oral contraception, most of which consists of ethinyl estradiol and a progestin, have a 24% increased risk of getting breast cancer, which decreases once they stop taking the pill (26).

## Role of Hormones in the Breast

The breast is a unique organ in that it develops primarily after birth, under the control of hormones (Figure 1) (27). A rudimentary ductal system present at birth begins to unfold during puberty and gains in complexity during adulthood with recurrent hormone stimulation during menstrual/estrous cycles. During pregnancy, ductal complexity increases further and finally secretory structures of saccular shape, called alveoli, bud all over the ductal system. Its embryonic-like state after birth makes the breast exquisitely plastic and particularly susceptible to carcinogenesis.

The mouse mammary gland has served as a model to study gene function in vivo and to genetically dissect gene function in development. A large number of mouse mutant strains are available, and tissue recombination experiments allow one to generate epithelial specific mutants (27). This approach has revealed that mammary epithelial intrinsic ER$\alpha$ signaling is required for pubertal ductal elongation (28). PR is essential in the mammary epithelium for side branching and alveologenesis (29), whereas the epithelial prolactin receptor is required for alveologenesis and milk secretion (Figure 1) (30).

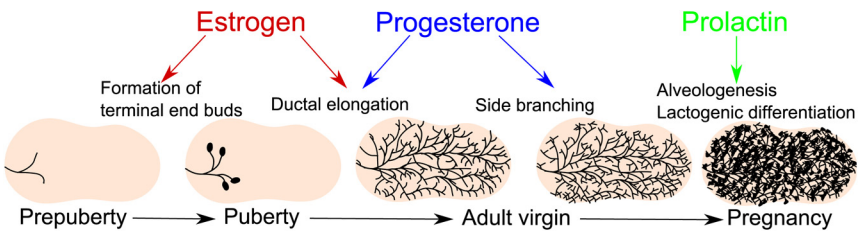


**Figure 1.** Mammary gland development in the mouse. Schematic representation of distinct stages of postnatal mammary gland development. In the pubertal mammary gland, terminal end buds appear at the tips of the ducts triggered by ovarian estrogens, which require epithelial ER. The ducts elongate and bifurcate until the edges of the fat pad are reached, which coincides with sexual maturity. Repeated stimulation with progesterone, as occurs during estrous cycles, results in the formation of side branches, which bud from preexisting ducts at a 90° angle. Side branch formation is blocked in $PR^{-/-}$ mammary epithelia. Ductal complexity continues to increase during the first half of pregnancy. In the last third of pregnancy, secretory structures of saccular shape, alveoli, sprout all over the ductal system and differentiate into milk-producing units under the control of prolactin receptor signaling.

On the one hand, different hormone receptor signaling pathways are limiting at distinct developmental stages. On the other hand, the mammary epithelium responds differently to a hormonal stimulus depending on its developmental stage. Hormone ablation and replacement experiments have shown that 17-$\beta$-estradiol induces cell proliferation specifically in pubertal (31) but not in adult mammary glands. In the adult, ie, more than 8-week-old, female mouse, 17-$\beta$-estradiol pretreatment induces the expression of PR (32), whereas subsequent stimulation with progesterone triggers proliferation (33). Hence, in the adult female PR signaling is the major stimulus of cell proliferation.

## Human Breast

The anatomy of the human breast with its 15–25 ducts that each give rise to a lobe containing multiple terminal ductal lobular units and 2 distinct stromal compartments, the intralobular and interlobular stroma, is more complex than that of the mouse mammary gland, which has a single stem ductal tree embedded in a homogeneous fatty stroma. Nevertheless, in terms of hormonal regulation, there seem to be substantial similarities across species. In most mammals, the ovaries first secrete estrogens in response to increased secretion of gonadotropins, and sexual maturity coincides with the establishment of cyclic peaks of ovarian progesterone secretion. Progesterone levels increase after ovulation when the body anticipates pregnancy, and continue to rise when pregnancy is established.

Pathologists observe proliferative activity in the breast epithelium during the luteal phase, when progesterone levels peak (Figure 2) (34, 35), suggesting that mouse and human mammary epithelia may indeed be similarly regulated, at least with regards to hormonal control of cell proliferation. Recently developed ex vivo models of the human breast have shown that progesterone elicits cell proliferation (36, 37). Of note, the dog, a species with particularly long luteal phase, is especially prone to mammary carcinoma (38)

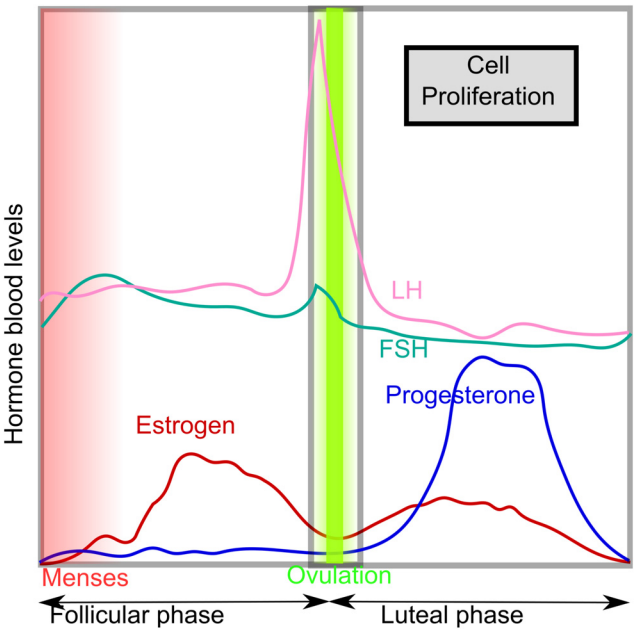


**Figure 2.** The human menstrual cycle. Graph showing serum levels of the major fluctuating hormones across a menstrual cycle. Note that progesterone levels peak during luteal phase. Estrogen reaches its maximum levels during follicular phase; a smaller peak follows during luteal phase. Cell proliferation is observed in the breast epithelium in the luteal phase and positively correlated with serum progesterone levels.

## Cellular and Molecular Mechanisms of Progesterone Action in the Mammary Epithelium

### Cell proliferation

The mammary epithelium is bilayered: the inner layer of luminal cells is surrounded by a meshwork of elongated myoepithelial cells, which are in close contact with the basal membrane. Luminal cells touch the lumen and are frequently opposed to the cells that are summarized under the term "basal cells": the subluminal, myoepithelial, progenitor, and stem cells. Between 30% and 50% of the luminal cells express ER in the adult female, whether rodent or human (39, 40). Because PR is an ER target gene, it is coexpressed in the same cells, although evidence has emerged that, at least in the human breast, PR is also independently expressed (41, 42). In the adult mammary epithelium, most cell proliferation occurs in the luminal compartment, but few of the proliferating cells express ER and PR (39). When mammary epithelial cells that are PR deficient (genetically $PR^{-/-}$) are grafted on their own to cleared mammary fat pads, they hardly proliferate in adult hosts. However, when the $PR^{-/-}$ mammary epithelial cells are intermingled with *PRwt* mammary epithelial cells in a 1 to 10 ratio, they proliferate and contribute to all aspects of mammary gland development in the context of the resulting chimeric epithelia (29), indicating that PR signaling can occur in a paracrine fashion. The same applies to $ER^{-/-}$ mammary epithelial cells, which, when grafted on their own, fail to proliferate at all, but which contribute to all aspects of mammary gland development in the context of chimeric epithelia (28). This motivates us to name the cells expressing ER and PR "sensor cells" (43), because they relay the systemic signal to local partners by emitting paracrine signals (Figure 3).

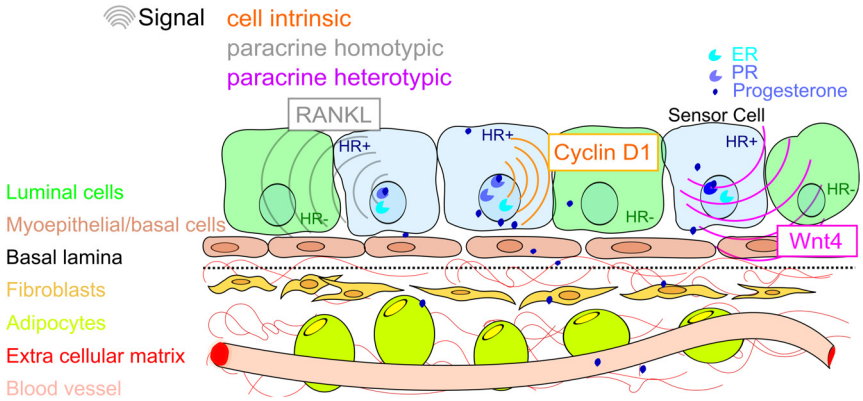


**Figure 3.** Signaling downstream of progesterone. Schematic representation of the bilayered mammary epithelium and the intra- and intercellular signaling activated by progesterone. An inner, luminal layer is surrounded by myoepithelial/basal cells, which are in contact with the basal lamina. Progesterone binds its receptor in a subset of HR+ luminal cells, the sensor cells (light blue). In certain PR+ cells, it induces cell proliferation by a Cyclin D1-dependent mechanism (cell-intrinsic signaling). It induces RANKL, which elicits cell proliferation in neighboring HR− cells (paracrine homotypic) and *wnt4*, which acts on myoepithelial cells (paracrine heterotypic) and increases stem cell activity.

When adult female mice are hormonally ablated and subsequently pretreated with estrogens, progesterone induces cell proliferation in 2 waves. During the first 24 hours, PR+ cells proliferate, whereas proliferation of PR− cells is observed subsequently (44). The first, small wave of cell-intrinsic proliferation requires cyclin D1; whether this relates to its cell cycle and/or its transcription-related functions is unclear. Support for such scenarios can be found in observations from the PR+ breast cancer cell line T47D, which reveal both that PR and CyclinD1 interact physically and are found in transcription complexes that bind to DNA and that down-modulation of CyclinD1 expression blocks PR-B-induced gene transcription (45). The second wave of cell proliferation, induced by a paracrine mechanism, is larger and relies on a tumor necrosis family member, receptor activator of NfκB ligand (RANKL). Progesterone increases RANKL mRNA expression by a posttranscriptional mechanism stabilizing the mRNA (36). RANKL protein is detected exclusively in PR+ cells (44, 46). Whether RANKL itself acts as mitogen, or removes a growth-inhibitory signal, or acts through a more complex loop involving other cell types, possibly infiltrating immune cells known to express the receptor, awaits further clarification. Although individual cells in luminal and abluminal locations express the cognate receptor RANK (47), it remains to carry out costainings to determine whether RANK+ cells are actively cycling epithelial cells.

## Stem cell activation

During luteal phase, stem cells are likely to be activated in anticipation of the cell number expansion of pregnancy. Stem cells have been studied by 2 major approaches, one entailing fluorescence-activated cell sorting, to enrich for cells with the ability to reconstitute mammary glands divested of their endogenous epithelium, and the other lineage tracing. Stem cells as defined by the first approach are located in the basal layer and express high levels of integrin $\beta 1$ and $\alpha 6$ (48, 49) and have been shown to expand in response to hormone stimulation (50, 51). Lineage-tracing experiments indicated, however, that most postnatal cell proliferation derives from luminally restricted stem cells (52, 53). To assess the role of PR signaling in stem cell function comprehensively, we resorted to serial transplantation. Mammary epithelium can reconstitute up to 7 transplant cycles (54). When we compared $PR^{-/-}$ and *PRwt* epithelia by serially transplanting them in contralateral glands, *PRwt* only slightly decreased in fat pad reconstitution over 4 generations, but $PR^{-/-}$ failed to reconstitute at the third generation, indicating that PR signaling is required to expand the stem cell pool during puberty and in adult life (55).

Wnt signaling is important to adult stem cells in many tissues, including the mammary gland (56), leading us to wonder whether *wnt4*, which we had previously identified as an important paracrine mediator of progesterone function in the mammary epithelium (57), affects stem cell function. Serial transplantation revealed that deletion of *wnt4* reduced regeneration potential more than deletion of *PR* did, indicating that *wnt4* is of central importance for regeneration potential and that *wnt4* has PR-independent functions. Indeed, *wnt4* is transcribed as early as postnatal day 5 in the mouse, ie, before detectable progesterone synthesis and PR expression in the mammary epithelium. Perinatal *wnt4* expression is independent of epithelial ER and PR signaling and is biologically relevant, as shown with a conditional allele in young animals, where it results in slightly delayed ductal outgrowth.

In the mammary epithelium of adult females, *wnt4* is exclusively transcribed in PR+ cells (55). Thus, *wnt4*, like RANKL, is a paracrine factor that is synthesized in PR+ cells and, as observed for RANKL, not all PR+ cells express *wnt4*.

Wnt4 can activate both canonical and noncanonical Wnt signaling (58, 59). An Axin2::LacZ reporter that reflects canonical Wnt signaling activity in multiple target tissues (60) revealed activity in the basal cells that correlates with *wnt4* expression, peaking in diestrous and in

early pregnancy. Deletion of either *wnt4* or *PR* in the mammary epithelium abrogates this activity, indicating that canonical Wnt signaling in the basal cells requires PR and *wnt4*.

Wnt4 emerges as central activator of mammary epithelial stem cells and of their niche(s). On the one hand, it may act directly on bipotent or basally restricted stem cells located in the basal layer, which can be identified based on expression of the protein c receptor, itself a Wnt target gene (61). In this, Wnt4 is helped by a membrane protein expressed in hormone receptor negative cells, R-spondin 1, which enhances canonical Wnt signaling and is itself induced by hormone stimulation (62). On the other hand, *wnt4* may act directly, possibly via noncanonical Wnt signaling and/or indirectly via distinct paracrine signals on luminally restricted stem cells. A potential paracrine mediator is GH, which can be synthesized in the breast epithelium and has been implicated in progesterone-induced activation of stem cells in the human breast, in work inspired by observations on dogs (63).

Two lines of work suggest that at least some of the findings in rodent models are of relevance to humans. First, work with a novel ex vivo model for the human breast consisting of tissue microstructures isolated from fresh reduction mammoplasty specimens that remain responsive to hormones, has shown that progesterone triggers cell proliferation in the adult human breast tissue and that it induces the expression of *RANKL* and *WNT4* transcripts (36, 37). Second, next-generation whole transcriptome sequencing was used to analyze global gene expression in the breast epithelium from 20 premenopausal women, who were not affected by breast cancer and donated breast tissue to the Susan G. Komen for the Cure Tissue Bank (http://komentissuebank.iu.edu/), and who were carefully staged for the menstrual cycle (64). This study revealed 255 genes that are differentially expressed between follicular and luteal phase, with 221 increased in luteal phase; in functional terms these genes related to cell cycle and mitosis, and DNA damage and repair, as also observed in vitro (65, 66). Interestingly, this unbiased approach identified 3 paracrine factors: RANKL, WNT4, and epiregulin (64).

## Additional Complexities

These findings suggest that PR signaling and its downstream effectors activate biological processes, such as cell proliferation and stem cell activation, that may account for the tumor-promoting effects of recurrent menstrual cycles. The same mechanisms may be activated when exogenous progestins are administered, as in the context of HRT and oral contraception. However, PR signaling itself is context-dependent, and not all PR signaling is tumor promoting. Pregnancies have a protective effect early in life with a 50% reduction in lifetime risk of breast cancer before the age of 20 (15). However, they bring on very high levels of progesterone, with serum progesterone reaching 180 ng $mL^{-1}$ in the third trimester, compared with 8–33 ng $mL^{-1}$ in luteal phase and 0.1–0.8 ng $mL^{-1}$ in follicular phase. Thus, the biological effects of progesterone may depend on the dose, the duration of the stimulus, the presence of concomitant high levels of 17-$\beta$-estradiol and other hormones, as well as on the woman's age.

A third ovarian hormone, testosterone, fluctuates to some extent during the menstrual cycle with a modest peak 3 days before the LH peak (67, 68). Interestingly, testosterone was reported to be the only hormone, the blood levels of which correlated with breast cancer risk in women with regular menstrual cycles (69). Whether cyclic activities of this hormone contribute to the risk associated with menstrual cycles needs to be explored. The role of this hormone in tumorigenesis is complex and dependent on the ER status of the tumor, as reviewed in Ref. 70.

A number of other hormones impinge on the basic regulatory network controlled by the ovarian hormones (71). They may serve to fine-tune the system or have distinct functions. In this context, an extensive study of normal human breast samples is of interest. It revealed 7 subsets of HR+ cells, all of which are luminal in the human breast: ER+, androgen receptor (AR)+, vitamin D receptor (VDR)+, ER+AR+, ER+VDR+, AR+VDR+, and ER+AR+VDR+. Other hormone receptors that were tested, including thyroid hormone receptor-$\alpha$, thyroid hormone receptor-$\beta$, parathyroid hormone 1 receptor, oxytocin receptor, various somatostatin receptors, RAR$\alpha$, RAR$\beta$, RXR$\alpha$, and RXR$\beta$, did not show a bimodal expression pattern (72). It will be of interest to see whether these populations of HR+ cells are conserved across species and whether the distinct receptor expression patterns characterize distinct cell types with specific biological function.

## Tumor-Promoting Action of Progesterone

Based on the above, we propose a model of menstrual cycle effects on breast carcinogenesis (Figure 4), in which the repeated activation of PR signaling during luteal phase may be tumor promoting. Some of the effects of progesterone are cell-intrinsic, but many biological responses rely on paracrine signaling that can be homotypic, ie, to neighboring luminal cells, or heterotypic, ie, to the myoepithelium and possibly to stromal cell types.

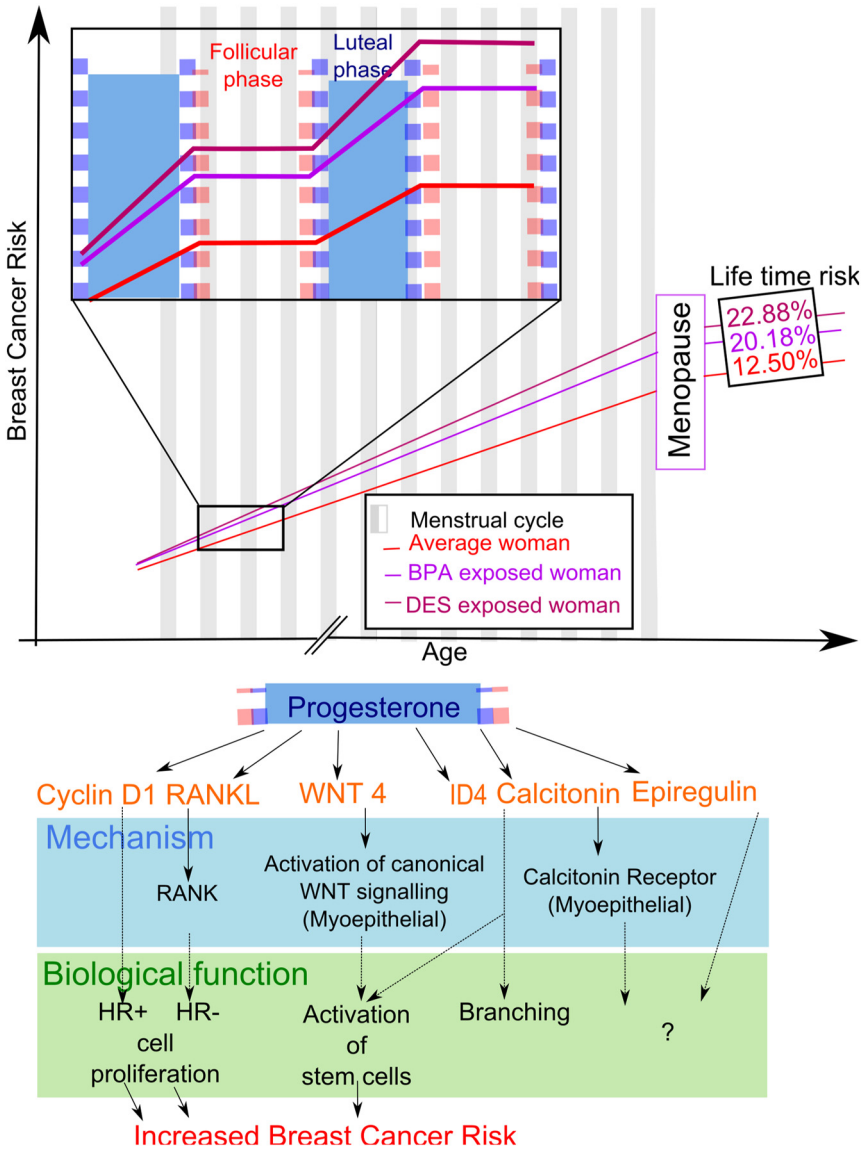


**Figure 4.** Model of how endocrine factors affect breast cancer risk. Graph showing breast cancer risk plotted over a woman's age, depending on whether or not she was exposed to DES or BPA. With each menstrual cycle, breast cancer risk increases through progesterone-induced events during luteal phase. The model proposes that perinatal exposure to endocrine disruptors increases the sensitivity of the breast to progesterone and hence increases the slope of the curve (top panel and inset). Various factors such as RANKL, WNT4, epiregulin, CyclinD1, ID4, and calcitonin, which act through distinct mechanisms and have been shown to have distinct biologically functions, have been implicated in the biological response to progesterone that may be amplified due to perinatal exposure.

Tumor-promoting effects of progesterone are also observed in rodents, where chemically (7,12-Dimethylbenzanthracene)-induced carcinogenesis is enhanced/accelerated by progesterone/progestin administration (73, 74). In support of this model, pharmacologically or genetically blocking RANKL delayed tumorigenesis (47, 75). Interestingly, RANKL inhibition was not effective anymore once the tumor was fully established (47), suggesting that the PR/RANKL axis is important specifically early in the pathogenesis of mammary carcinomas.

Similarly, the Wnt signaling pathway may promote tumorigenesis. In the mouse, *wnt1*, a *wnt4* cousin, was long identified as an oncogene by cloning of the frequent insertion site of the oncogenic mouse mammary tumor virus (76). Ectopic expression of Wnt1 in the mammary epithelium results in highly penetrant widespread hyperplasia and, ultimately, tumors (77), consistent with an early tumor-promoting effect that may rely largely on indirect and niche-related effects. In T47D cells, Wnt1 is a PR-B target and induces matrix metalloproteases to shed epidermal growth factor receptor ligands that transactivate the epidermal growth factor receptor (78).

## Endocrine Disruption

The highly complex network of hormones that orchestrates the development of the mammary gland through different phases of its life cycle is still incompletely understood. There are concerns that endocrine-disrupting factors are implicated in breast carcinogenesis, because cancer incidence in other hormone-sensitive organs, such as the prostate and the testicles are also on the increase. Determining the role of endocrine-disrupting compounds in breast carcinogenesis is an enormous challenge; these compounds penetrate all aspects of our daily lives, so that exposures are hard to define and impossible to trace. In addition, timing of exposure is a major issue, because hormone action is highly dependent on developmental stages.

As breast cancer is highly prevalent and multifactorial, it is impossible to attribute specific cases to exposures, which are poorly defined. Nevertheless, we would like to attempt to assess the effect of exposure to BPA, which has made its way into consumer products and is detected in body fluids of more than 90% of the population (79).

To assess whether perinatal exposure to BPA affects mammary gland development, we added concentrations

ranging from the environmentally relevant 0.6 μg/kg per body weight/d to 1.2 mg/kg per bw/d to the drinking water of breeding C57Bl6 mice. Hence, the females under observation were exposed to BPA in utero through their mothers and during the first 3 weeks of their lives, when the mother was nursing them, before they were weaned into a BPA-free environment. As described in rats and CD1 mice (80–82), more terminal end buds were observed during puberty; interestingly, at the lowest concentrations of BPA (83). To assess whether the predominantly progesterone-controlled adult mammary epithelium was affected, we assessed cell proliferation. Because subtle increases in cell proliferation indexes are impossible to discern given the interindividual variation and the imprecision of the current available assays, we opted for counting cell numbers in 3-month-old females. The numbers obtained would reflect accumulated changes due to slightly increased proliferation rates during each estrous cycle. After ascertaining by fluorescence-activated cell sorting analysis that there were no changes in the ratio of different cell types in mammary glands from exposed and unexposed females of the same age, cell numbers were determined. In BPA-exposed animals, cell numbers were on average 1.5-fold increased. A control group of females, which had been exposed to diethylstilbestrol (DES), in the same way showed a 1.7-fold increase in cell number (83).

In order to extrapolate these observations to humans, let us assume that perinatal exposure is critical during in utero development (rather than during the nursing period) and that BPA and DES display analogous behavior with regard to cell proliferation in mice and breast cancer risk in women. If women exposed to DES in utero have 1.83× increased relative risk of breast cancer when they are over 40 years old (84), we predict (based on the 1.5- vs 1.7-fold increase in cell numbers) that perinatal BPA exposure as it now occurs in most of the population will increase relative risk 1.6-fold. Because breast cancer affects 1 in 8 women, ie, 12.5% of women, this means that 20%, ie, 1 in 5 women could be affected in the future (Figure 4).

A potential mechanism underlying the increased sensitivity to progesterone is an increase in the number of PR+ cells that is reflected in increased induction of RANKL and *wnt4* in response to ex vivo progesterone stimulation (83). Hence, perinatal exposure to endocrine disruptors that mimic estrogens like BPA may increase breast cancer risk by increasing the sensitivity of the mammary epithelium to progesterone and amplifying the biological response during each menstrual cycle (Figure 4).

Clearly, the mechanisms of endocrine disruption are more complex, because BPA acts at multiple levels on mammary gland development (85). We have a long way to go to say in which way findings in C57Bl6 mice are relevant to human health; yet, at this point, we cannot exclude that current exposures may result in more breast cancer cases. Efforts should be made to broaden the definition of carcinogen to account for the biological complexities of hormone regulation that are so important for homeostasis and hence our wellbeing, in particular the epigenetic changes that hormones elicit, which can result in permanent remodeling of the chromatin with transgenerational effects (86, 87).

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