The role of steroid hormones in breast cancer stem cells

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Abstract

Breast cancer stem cells (BCSCs) are potent tumor-initiating cells in breast cancer, the most common cancer among women. BCSCs have been suggested to play a key role in tumor initiation which can lead to disease progression and formation of metastases. Moreover, BCSCs are thought to be the unit of selection for therapy-resistant clones since they survive conventional treatments, such as chemotherapy, irradiation, and hormonal therapy.

The importance of the role of hormones for both normal mammary gland and breast cancer development is well established, but it was not until recently that the effects of hormones on BCSCs have been investigated. This review will discuss recent studies highlighting how ovarian steroid hormones estrogen and progesterone, as well as therapies against them, can regulate BCSC activity.

Key Words
- breast
- mammary gland
- progesterone receptor
- estrogen receptor
- cancer stem cells

Breast cancer stem cells

The cancer stem cell (CSC) theory proposes a hierarchical organization of the cells within a tumor, where only a small subset of cells, the CSCs, is believed to drive and sustain tumor growth. CSCs are defined as self-renewing tumor-initiating cells (TICs), which would implicate them in tumor relapse and resistance to therapy, making them an important therapeutic target (Reya et al. 2001).

The first report establishing the presence of breast CSCs (BCSCs) discovered that CD44⁺CD24⁻/lowESA⁺ lineage⁻ (named CD44⁺CD24⁻ henceforth) cells, isolated from human breast tumors by FACS, were enriched for tumor-initiating capacity in immuno-compromised mice (Al-Hajj et al. 2003). CD44⁺CD24⁻ cells can be serially passaged and form tumors containing both tumorigenic cells (CD44⁺CD24⁻) and non-tumorigenic cells. Breast cancers with high levels of CD44 and low levels of CD24 have been associated with the triple negative phenotype (i.e. lacking estrogen receptor (ER), progesterone receptor (PR), and HER2 expression) and inferior overall survival (Liu et al. 2007, Honeth et al. 2008).

Besides isolation of CD44⁺CD24⁻ cells, other strategies have been used to identify populations enriched for BCSC activity. Mammosphere formation, high aldehyde dehydrogenase (ALDH) activity, capacity to retain PKH26 dye or ability to efflux lipophilic dyes (side population (SP)), are all examples of properties that have been used to isolate these TICs. The mammosphere colony assay relies on the ability of BCSCs to survive in non-adherent serum-free culture conditions and form individual spherical colonies, called mammospheres (Dontu et al. 2003, Ponti et al. 2005, Farnie et al. 2007). On the other hand, the activity of ALDH1, which oxidizes intracellular aldehydes, is detected by an enzymatic assay (ALDE-FLUOR) and flow cytometric analysis (Ginestier et al. 2007). The proportion of cells expressing ALDH1 in breast tumors has been shown to correlate with poor clinical outcome (Ginestier et al. 2007, Charafe-Jauffret et al. 2010).
The PKH26 dye, which labels quiescent cells, has also been used to identify BCSCs in primary breast tumors by FACS sorting cells expressing CD49f, DLL1, and DNER (Pece et al. 2010). Hoechst dye exclusion activity has also been described as a method to identify a cellular fraction termed the SP that contains tumorigenic stem/progenitor cells (Patrawala et al. 2005). Finally, an autofluorescent epithelial CSC phenotype has recently been reported, however it still remains to be proven whether it can be used to identify BCSCs (Miranda-Lorenzo et al. 2014).

There remains a lack of consensus as to the most robust method for the purification of BCSCs. The establishment of bona fide BCSC markers is hindered by breast cancer intra-tumor and inter-tumor heterogeneity of its cell populations. Nevertheless, the two most widely used cell populations to enrich for BCSCs are CD44+CD24− and ALDH+. A recent study reported that these two cell populations identify BCSCs in different states with gene expression profiles resembling cells with either mesenchymal (CD44+CD24− cells) or epithelial characteristics (ALDH+ cells) (Liu et al. 2014). Moreover, this study identified a small overlapping population of cells that is both CD44+CD24− and ALDH+, and suggested that BCSCs display cellular plasticity by dynamically switching between the mesenchymal and epithelial states. This epithelial–mesenchymal transition or vice-versa (mesenchymal–epithelial transition) is believed to be determined by the tumor microenvironment, with factors like hypoxia or transforming growth factor beta playing a key role in this process (Thiery 2002, Yang et al. 2008). It is feasible that other signaling factors that have been reported to modulate BCSC activity, such as hormones, may also influence this dynamic state.

In this review, we will discuss what is known about the regulation of BCSC function by the steroid hormones estrogen and progesterone and their antagonists.

**Estrogen and BCSCs**

Estrogen is essential for the development of normal breast epithelium by promoting epithelial cell proliferation and ductal morphogenesis but also plays an important role in the growth of most breast cancers through their expression of ER (Bocchinfuso & Korach 1997, Colditz 1998). Epidemiological evidence suggests that breast cancer risk is positively associated with post-menopausal levels of estrogen (Clemons & Goss 2001). Estrogen effects are mainly mediated through binding to two nuclear ligand-activated transcription factors, the ERs ERα and ERβ, which then bind estrogen-responsive elements in the DNA to regulate the transcription of target genes (Yager & Davidson 2006). In the normal breast, ERα is found in luminal epithelial cells, but not in the stroma, whereas ERβ has been shown to be expressed in both luminal and myoepithelial cells, as well as stromal cells, such as fibroblasts and endothelial cells (Petersen et al. 1987, Spears et al. 2002). ERα, which has a higher affinity to the physiological form of estrogen, 17ß-estradiol, than ERβ, has been shown to be the major mediator of estrogen action (Bocchinfuso & Korach 1997, Kuiper et al. 1998). ERα (named ER henceforth) is a key regulator of breast cancer and its expression status is currently used together with other receptors in the classification of breast cancer subtypes. ERα+ tumors are strongly associated with the luminal subtype and are generally characterized by expression of luminal differentiation markers (Perou et al. 2000).

Although the importance of estrogen in breast cancer is well established, the effects of estrogen on BCSCs are not fully understood and are still a matter of debate (Simões & Vivanco 2011). Estrogen may exert influence on stem cells via paracrine mechanisms because CD44+CD24− and ALDH+ CSCs have been shown to lack expression of ER or express it at very low levels (Morimoto et al. 2009, Harrison et al. 2013, Simões et al. 2015). Similar to what happens in the normal mammary gland, it has been suggested that estrogen can promote CSC activity of ERα+ BCSCs by inducing the secretion of paracrine growth factors from ERα+ cells. Fibroblast growth factor (FGF)/Tbx3 signalling, as well as epidermal growth factor (EGF) and Notch receptor signalling pathways, have been reported to control this paracrine mechanism and induce the expansion of CD44+CD24− CSCs (Fillmore et al. 2010, Harrison et al. 2013). In contrast to these findings, estrogen was shown to reduce the self-renewal capacity of MCF7 BCSCs by promoting differentiation through down-regulation of embryonic stem cell genes NANOG, OCT4, and SOX2 (Simões et al. 2011). These contradictory results may be due to differences in the methods used in these studies. Fillmore et al. and Harrison et al. exposed breast cancer cells grown in monolayer adherent culture (not enriched for CSCs) to estrogen whereas Simões et al. challenged BCSCs with estrogen by growing cells in non-adherent mammosphere culture conditions. Therefore, opposing effects of estrogen on CSC activity seem to be determined by the context in which the cells are cultured and by the analysis of different breast cancer cell populations.

The role of estrogen in clinical breast carcinogenesis is also contradictory. Whereas high levels of endogenous
estrogens increase the risk of postmenopausal breast cancer, randomised trials of exogenous estrogen alone (hormone replacement therapy) show it to reduce the incidence and mortality of breast cancer (Women’s Health Initiative) (LaCroix et al. 2011). This finding is similar to animal models where short-term treatment with pregnancy levels of estrogen can prevent the formation of mammary tumors (Rajkumar et al. 2001). This anti-cancer effect of estrogen has been suggested to explain the breast cancer preventative potential of early full-term pregnancy to lifetime breast cancer risk, although this cannot be attributed solely to estrogen levels given the complexity of pregnancy associated endocrine perturbation (Medina 2004). Hypothetically, the protective effect of estrogen may be due to breast stem cell differentiation during pregnancy and lactation, which would reduce the number of stem cells that could be precursors of cancer (Russo et al. 2005, Simões & Vivanco 2011). To add further complexity to the role of estrogen in breast cancer, higher doses have been used for many years to treat advanced disease, with response rates similar to those seen with the anti-estrogens (Ellis et al. 2009, Lewis-Wambi & Jordan 2009). Without doubt, more studies are needed to explore the complexities of estrogen signalling, stem cells and breast cancer risk and progression.

Endocrine resistance: biomarkers, up-regulated pathways, and BCSCs

Around 75% of breast cancers express ER and are treated with anti-estrogen adjuvant therapies to suppress ER mediated estrogen signaling and, therefore, inhibit proliferation of ER+ breast cancer cells (Ali & Coombes 2002). There are three main classes of anti-estrogen drugs that target and modulate ER activity: selective ER modulators (SERMs), aromatase inhibitors (AIs), and selective ER down-regulators (SERDs). The most common and successful SERM is tamoxifen, which prevents the effects of estrogen by competing for the ER ligand-binding site (Shiau et al. 1998). AIs block the function of aromatase, the enzyme that catalyses the last step of estrogen biosynthesis (Mokbel 2002). Tamoxifen and AIs are the endocrine therapies of choice in the adjuvant treatment of premenopausal and postmenopausal women respectively (Beelen et al. 2012). These and other anti-estrogens, such as the SERD fulvestrant, which binds ER and targets it for degradation through ubiquitination, are used sequentially in advanced breast cancer (Howell et al. 2004). Endocrine sensitivity can partly be predicted by serial analysis of the proliferation marker Ki67 expression in pre-surgical ‘window’ studies or longer term neoadjuvant studies of several months of treatment (Dowsett et al. 2011). More recently, a four-gene signature including genes related to immune signalling (IL6ST), apoptosis (NGFRAP1), and proliferation (ASPM and MCM4) was reported to predict the clinical response of patients treated with AIs (Turnbull et al. 2015). However, despite the undoubted success of tamoxifen (or similar endocrine) treatment, at least half of patients with micrometastatic disease will relapse despite therapy, often many years after initial surgery and endocrine therapy is completed (Early Breast Cancer Trialists’ Collaborative Group et al. 2011).

Such endocrine resistance compromises this otherwise effective treatment and thus the potential cure of ER+ breast cancers. Therefore, defining the mechanisms of endocrine resistance is a major research focus. Activation of classical signalling pathways, including the ones induced by HER2 and EGF receptor (EGFR), MAPK, and PI3K/AKT have been implicated in hormone resistance (Musgrove & Sutherland 2009). However, the only approved targeted therapies to improve outcomes of endocrine-resistant ER+ HER2− breast cancers are the mTOR inhibitor everolimus and the CDK4/6 inhibitor palbociclib combined with an AI or fulvestrant (Baselga et al. 2012, Finn et al. 2015, Turner et al. 2015). Therefore, a better understanding of the molecular changes associated with endocrine resistant growth is urgently needed to find treatments that can inhibit or delay the emergence of resistance.

BCSCs, which can survive for long periods in a dormant state, may be associated with tumor recurrence and metastases. These cells have been shown to be more resistant to chemo- and radio-therapies than non-CSCs (Phillips et al. 2006, Li et al. 2008). In endocrine therapy, accumulating evidence suggests that there is an increase in BCSCs in ER+ breast cancer following anti-estrogen treatment. Two studies have reported enrichment for cells with both BCSC gene and marker expression in breast tumor tissue following short term AI (letrozole) or tamoxifen treatment (Creighton et al. 2010, Kabos et al. 2011). Additionally, other studies demonstrated similar effects in ER+ breast cancer cell lines. For example, tamoxifen treatment increased both the number of mammospheres and the expression of NANOG, OCT4, and SOX2 in MCF7 breast cancer cells (Simões et al. 2011, Piva et al. 2014). MCF7 mammospheres were also shown to be resistant to high doses of tamoxifen (Cariati et al. 2008). Moreover, tamoxifen, fulvestrant, or estrogen deprivation increased the percentage of cells expressing cytokertatin 5 (CK5), a marker of human breast stem/progenitor cells also found in BCSCs, in T47D breast cancer cells (Creighton et al. 2009).
et al. 2010, Kabos et al. 2011). These data confirm that, while endocrine therapies target the differentiated proliferative breast cancer cells, they cannot effectively target the BCSCs.

Stem cell activity in ER$^+$ tumors is mainly due to a minority population of ER$^-$ cells, which cannot be directly targeted by anti-estrogens and therefore might be responsible for resistance and recurrence (Harrison et al. 2013, Simões et al. 2015). Indeed, circulating tumor cells of ER$^+$ primary tumors are in general found to be ER$^-$ (Fehm et al. 2009). In the clinic, ER negativity is associated with poor prognosis, precluding a response to all categories of anti-estrogen treatment and associating with a more aggressive and proliferative phenotype. Interestingly, expression of putative regulators of ER$^-$ BCSC activity like EGFR (Harrison et al. 2013), HER2 (Ithimakin et al. 2013), and FGF receptor (Fillmore et al. 2010), potentially resulting from selection of cells with a more stem-like phenotype have been associated with acquisition of endocrine resistance (McClelland et al. 2001, Hutcheson et al. 2003, Knowlden et al. 2003). Recently, the ER splice variant Erz36, which lacks both transactivation domains AF1 and AF2, was associated with BCSC regulation and endocrine resistance (Wang et al. 2005, Deng et al. 2014). Specifically, Deng et al. showed Erz36 to be essential for CD44$^+$CD24$^-$ BCSC enrichment induced by tamoxifen or fulvestrant. Erz36 is reported to be located in the cellular membrane and cytoplasm, and to rapidly activate MAPK/ERK signalling in the presence of estrogen. However, future studies are needed to better understand the importance of Erz36 isoform in BCSCs maintenance (Wang et al. 2006).

The potential involvement of BCSCs in endocrine resistance makes it imperative to understand the cellular signalling pathways that could be targeted to eradicate BCSCs and provide long-term disease-free survival. It has been established that these cells are dependent upon developmental signalling pathways, which may provide suitable targets for therapeutic intervention (reviewed in Visvader & Lindeman 2012). For example, activation of Wnt signalling due to high expression levels of stem cell marker SOX2 has been reported as an important tamoxifen-resistance mechanism (Piva et al. 2014). Another strong candidate for endocrine-resistant CSC regulation is the Notch pathway, which comprises four different transmembrane receptors (Notch1–4), five known surface-bound ligands (Delta-like 1, Delta-like 3, Delta-like 4, Jagged 1, and Jagged 2) and multiple transcriptional targets, including the Hes and Hey family of genes (Brennan & Brown 2003). It was previously shown that aberrant Notch activation is found in human breast cancers and correlates with recurrence within 5 years in ductal carcinoma in situ (DCIS) lesions (Stylianou et al. 2006, Farnie et al. 2007). Moreover, it was established that inhibition of the Notch signalling pathway reduces BCSC activity, and that the Notch4 receptor has a key role in controlling BCSCs (Harrison et al. 2010). Recently, our group demonstrated that treating ER$^+$ breast cancer cells with endocrine therapies leads to increased Jag1–Notch4 signalling and that combining endocrine therapies with a Notch pathway inhibitor can prevent BCSC enrichment induced by endocrine therapies (Simões et al. 2015). Our results suggest that inhibition of Notch signalling can help overcoming endocrine therapy resistance and might prevent recurrence in ER$^+$ breast cancer. Importantly, we also showed that both Notch4 activation and high expression of BCSC marker ALDH1 in patient primary tumors are predictors of resistance to endocrine treatments (Simões et al. 2015).

In summary, we speculate that BCSCs evade endocrine therapies, lie dormant and eventually re-initiate tumors in metastatic sites after treatment. Thus, BCSC-targeted therapies in combination with established anti-estrogens are likely to improve outcomes for breast cancer patients.

**Progesterone and BCSCs**

Progesterone has been shown to be vital for both pubertal side branching and lobular alveolar development of the mammary gland during pregnancy (Lydon et al. 1995, Brisken 2013). Importantly, in premenopausal women breast epithelial cell proliferation is highest in the progesterone dominant luteal phase of the menstrual cycle (Potten et al. 1988, Navarrete et al. 2005). Studies in mice have shown that mammary gland development results from progesterone-induced expansion of the mammary stem cell pool and have also shown that PR is important for carcinogen-induced mammary tumor formation (Lydon et al. 1999, Asselin-Labat et al. 2010, Joshi et al. 2010). In normal human breast cells, progesterone stimulation in matrix-embedded culture increased bipotent progenitor cell numbers (Graham et al. 2009).

The progesterone signal is mediated by the PR, which comprises two isoforms (PRA and PRB) that are only differentiated by a third activation function domain on the 5’ end of PRB (Kastner et al. 1990). The two isoforms are generally co-expressed at similar levels in the normal breast but the ratio can be altered in human breast tumors, resulting in a predominance of one particular isoform, usually PRA, over its counterpart (Graham et al. 2005, 2009).
Isoform-specific mouse mutants reveal that PRB is the functionally important form in mammary gland morphogenesis, whereas PRA is important for ovarian function (Mulac-Jericevic et al. 2000, 2003). These isoforms display only partially overlapping transcriptional signatures with PRB modulating expression of significantly more genes than PRA (Richer et al. 2002). Relative loss of PRB is seen with the development of atypia or malignancy and in women with germline mutations in BRCA1 or BRCA2 (Mote et al. 2002). Interestingly, women with such mutations have double the serum progesterone levels compared to age matched WT controls although the significance of this finding is not known (Widschwendter et al. 2013).

In the normal mammary tissue, progesterone-induced gland expansion is mediated through paracrine proliferative signals, including receptor activator of nuclear factor-kappa B ligand (RANKL) and WNT4, secreted from PR+ sensor cells and acting on PR− stem cells, expressing the RANK receptor and Wnt receptors, such as Frizzled (FZD) and LRP5/6 (Graham et al. 2009, Gonzalez-Suarez et al. 2010, Joshi et al. 2010). In multiple rodent models, deletion or inhibition of PR or the RANK/RANKL pathway results in significant reduction in mammary carcinogenesis (Lydon et al. 1999, Poole et al. 2006, Gonzalez-Suarez et al. 2010, Schramek et al. 2010). Recent evidence has established CXCRI4 receptor and its ligand CXCL12 as potential key mediators of progesterone-induced stem/progenitor cell functions in normal mammary gland (Shiah et al. 2015). CXCL12 is localized on PR+ luminal cells whereas CXCRI4 is induced by progesterone in both basal and luminal PR− cells. Significantly, Shiah et al. showed that inhibition of CXCRI4-CXCL12 signalling is able to arrest the progesterone-induced expansion of mammary stem/progenitor cells. Finally, it has been demonstrated recently that progesterone induces growth hormone (GH) secretion in human breast epithelial cells, which increases proliferation of GH receptor (GHR) positive stem/progenitor breast cells (Lombardi et al. 2014).

In an analogous manner progesterone has been shown to expand the population of BCSCs in breast cancer cell lines. In particular, progesterone was shown to increase the population of CK5+ and CD44hi or CD44+CD24− BCSCs in several ER+PR+ cell lines but particularly in T47D cells, which express high levels of PR even in the absence of estrogen (Axlund et al. 2013, Finlay-Schultz et al. 2014, Hilton et al. 2014). Importantly, in cell lines where PR expression is dependent on estrogen, cells need to be treated with estrogen and progesterone, while estrogen alone was not able to induce BCSCs.

The mechanisms behind the progesterone-induced expansion of BCSCs have not been fully elucidated. However, progesterone treatment of cell lines has been shown to repress miR-29 and miR-141, de-repressing KLF4 and STAT5A respectively (Cittelly et al. 2013, Finlay-Schultz et al. 2014). In both studies, this resulted in expansion of the CK5+/CD44+ CSC population and enhancement of colony formation and tumor initiating capacity. KLF4 is a transcription factor required for maintenance of both BCSCs (Yu et al. 2011) and pluripotency in embryonic stem cells (Zhang et al. 2010) whereas STAT5A is a transcription factor that regulates the mammary luminal progenitor population (Yamaji et al. 2009). BCL6, which appears to be critical in the maintenance of some leukaemic stem cells, was also reported to be essential for progesterone-induction of CK5+ cells (Hurtz et al. 2011, Sato et al. 2014). Interestingly, the progesterone-induced expression of BCL6 was inhibited by prolactin, further demonstrating the complex interplay between hormonal signalling axes in the regulation of BCSCs (Sato et al. 2014). It is also possible that PR+ cells communicate with PR− BCSCs through similar paracrine pathways as in the normal mammary gland. Indeed, non-endogenous overexpression of RANK in human breast cell lines induces stemness by increasing the CD44+CD24− BCSC population, promoting tumour initiation and metastasis (Palafox et al. 2012). However, clinical trials of the RANKL inhibitor denosumab do not show any improvement in cancer control or survival despite their valuable role in reducing skeletal complications from bone metastases. In summary, this evidence suggests that progesterone is responsible for the expansion of both normal and breast cancer stem cells but that the precise mechanisms may be divergent. However, both the PR itself and some of the paracrine/downstream signals described are targetable and may hold promise as breast cancer therapies.

Anti-progesterone drugs and BCSCs

Women’s Health Initiative study reports that combination of estrogen with progesterin (synthetic progesterone derivative), but not estrogen alone was associated with greater breast cancer incidence and mortality (Chlebowski et al. 2010). The progesterone role in mammary tumorigenesis may be explained by the expansion of stem cell populations, which are likely to originate BCSCs and lead to the formation of ER+/PR+ tumors (Narod 2011).

Despite much promise in the early 1990s, no anti-progesterin is a recommended standard of care in
anti-cancer treatment. However, there is a renewed interest in anti-progestin drugs indicated by several current clinical trials using mifepristone and onapristone in breast cancer and other solid tumors (see NCT01493310, NCT02014337, NCT02046421, NCT02049190, and NCT02052128 on US clinical trials database, https://clinicaltrials.gov/). Based on recent research, it is possible that these drugs target BCSCs in ER$^+$/PR$^+$ tumors, although this remains hypothetical and merits further investigation.

Conclusions

The published data suggests that in breast cancer both estrogen and progesterone signalling have multifarious effects on CSC activity. Since BCSCs are reported to be low or negative for steroid hormone receptors, the effects are likely to be mainly indirect, transmitted through paracrine or juxtacrine cell–cell signalling pathways (Fig. 1). We do not exclude the possibility that there is some autocrine signalling downstream of hormones that may contribute to regulation of BCSCs. The effects of estrogen and progesterone have only been partially described in cancer tissues. For progesterone in particular there is more data from normal mammary epithelium than from cancer tissues. For estrogen, there are reports that following in vitro treatment of serum-starved breast cancer cells, CSC activity is stimulated and that this requires regulation by EGF, FGF, or Notch1 receptors, suggesting indirect, paracrine or juxtacrine signalling between cells (Fig. 1). On the other hand, anti-estrogens, such as tamoxifen or fulvestrant, block direct effects of estrogens on cell growth, and the indirect signals to the ER$^-$ BCSCs. Paradoxically however, tamoxifen has been demonstrated to increase...
BCSC activity in mammosphere colony culture (Simões et al. 2011, Piva et al. 2014), and more recently, the same has been confirmed for both tamoxifen and fulvestrant in vivo (Simões et al. 2015). The data suggest that while anti-estrogens are cytostatic for the ER+ cells, there is an increase in the proportion of ER− BCSCs and their activity. This increase could be due to selective enrichment by treatment, by induction of a change in cellular phenotype from ER+ non-BCSC to ER− BCSC, or possibly a combination of both of these effects. Whatever the reason, the mechanism for the increase induced by anti-estrogens is reported to be Jag1–Notch4 signalling between ER− BCSCs (Simões et al. 2015), rather than the signals from the ER+ cell shown here (Fig. 1).

For progesterone, the data are clear it has a role in regulating the expansion of normal mammary stem and progenitor cells through several signalling pathways including CXCL12/CXCR4, GH/GHR, WNT4/Fzd, and RANKL/RANK. In breast cancer, there are cell line data suggesting that progesterone may regulate BCSCs but the importance of the previous signalling networks is not established (Fig. 1). Since progesterone does not directly stimulate proliferation in most breast cancers, the role for anti-progesterone drugs in breast cancer may be to abrogate progesterone effects on BCSC activities, although this is yet to be proven.

In summary, the data accumulated thus far indicate that estrogen and progesterone have mostly indirect effects on BCSCs since they are mainly ER+ and PR− cells. Results from both normal and malignant epithelial cell–cell interactions suggest that estrogen and progesterone elicit these effects through different paracrine/juxtacrine regulatory pathways. Finally, since there are several putative pathways downstream of each estrogen and progesterone, there will be interactions and redundancy between these, yielding a subtle complexity in the consequences for the BCSC.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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