

Breast Cancer Prevention: Lessons to be Learned from Mechanisms of Early Pregnancy-Mediated Breast Cancer Protection

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Abstract

Pregnancy at early, but not late age, has a strong and life-long protective effect against breast cancer. The expected overall increase in breast cancer incidence demands the development of a pharmaceutical mimicry of early-age pregnancy-mediated protection. Recently, converging results from rodent models and women on molecular and cellular mechanisms underlying the protective effect of

early-age pregnancy have opened the door for translational studies on pharmacologic prevention against breast cancer. In particular, alterations in Wnt and TGF β signaling in mammary stem/progenitor cells reveal new potential targets for preventive interventions, and thus might help to significantly reduce the incidence of breast cancer in the future. *Cancer Res*; 75(5); 803–7. ©2015 AACR.

The World Health Organization estimates that the annual death-toll of breast cancer will increase by more than 60% during the next 20 years (1). This expected dramatic increase in breast cancer incidence demands the development of new effective prevention strategies. Because early- but not late-age pregnancy significantly reduces the life-long breast cancer risk of women (2), pharmaceutical mimicry of the breast cancer-protective effect of early-age pregnancy promises to be of considerable help in future reduction of breast cancer-related mortality. To develop such pharmacologic prevention strategies against breast cancer, the cellular and molecular mechanisms underlying the protective effect of early-age pregnancy must be understood. In this regard, considerable progress has been made recently by the demonstration that early-age pregnancy changes cell fate determining signaling pathways preferentially in mammary stem/progenitor cells (3, 4). Although several challenges remain before successful pharmacologic prevention against breast cancer can be achieved, the ultimate goal must be the translation of these underlying processes into methods to bring about the protective effect of pregnancy at early age in women without the need for teenage pregnancies.

Pregnancy and Risk of Breast Cancer

Numerous epidemiologic studies have identified pregnancy as the most significant modifiable factor for the risk of breast cancer in women (2). After a transient increase in breast cancer risk immediately following parturition, pregnancy at an early age leads to a strong and life-long protective effect against breast cancer. Women who have gone through pregnancy before the age of 20 years

develop 50% fewer breast cancers compared with nulliparous women. However, as the age at first full-term pregnancy increases, the breast cancer-protective effect of pregnancy decreases: For first full-term pregnancies between the ages of 30 and 34 years, pregnancy-induced breast cancer protection is negligible. For first full-term pregnancies after the age of 35 years, even an increase in the overall breast cancer risk is observed (2). Regarding different breast cancer subtypes, epidemiologic data show that early-age pregnancy specifically protects against estrogen receptor- and progesterone receptor-positive (ER⁺/PR⁺) breast cancers with no effects on the risk of ER and PR negative (ER⁻/PR⁻) breast cancers (5). The specific protection provided by early-age pregnancy against ER⁺/PR⁺ breast cancers results in an increase in the observed rates of breast cancer lethality in women with early-age childbirth due to selection of patients with more malignant ER⁻/PR⁻ breast cancers (6, 7). A similar cancer-protective effect of pregnancy is observed in rodents. In rats and mice, carcinogen administration leads to approximately 75% fewer incidences of mammary cancers in parous as compared with nulliparous control animals (8). Hormonal mimicry of pregnancy by treatment with estrogen and progesterone or human chorionic gonadotropin for 21 days has a similar protective effect as pregnancy itself in rodent models (8). Also, rodent models have been successfully used to mimic the effects of pregnancy concentrations of 17 β -estradiol in human breast tissue transplants (9). These findings demonstrate that rodents can be used as reliable model systems to test hypothetical cellular and molecular mechanisms underlying the breast cancer-protective effect of early-age pregnancy in women.

Possible Mechanisms of the Protective Effect of Early-Age Pregnancy against Breast Cancer

Hypothetical mechanisms for the breast cancer-protective effect of an early full-term pregnancy in both humans and rodents include alterations in systemic hormone levels as well as in local cellular processes in the mammary gland (10). However, systemic hormonal changes alone are unlikely to explain the full breast cancer-protective effect of early-age pregnancy, as they are

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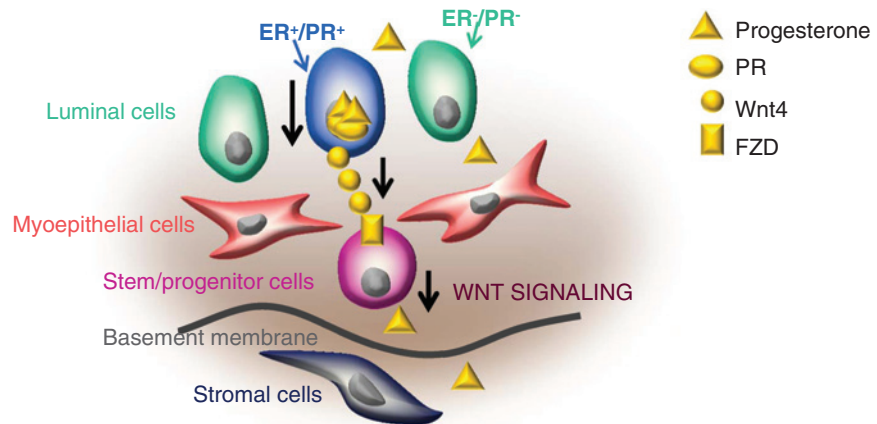
generally associated with local cellular alterations. Furthermore, respective studies have produced inconsistent results (11, 12). More convincing are hypotheses that localize breast cancer-protective mechanisms to cellular processes of the mammary gland. They include early pregnancy-induced changes in (i) organ differentiation, (ii) hormone responsiveness, (iii) proapoptotic pathways, (iv) extracellular matrix (ECM) or stroma, and (v) mammary stem cell fate. Alterations in organ differentiation are suggested by studies of genome-wide expression profiles in lobular breast tissues of women or entire mammary glands of rodents, which have shown increased expression of differentiation genes after parity (13, 14). However, because differentiation-inducing agents such as the prolactin-like molecule placental lactogen or the dopamine receptor antagonist perphenazine fail to protect against mammary tumors in rodents, differentiation *per se* cannot explain the full breast cancer-protective effect of early-age pregnancy in rodents (8). The possibility for a role of altered hormone responsiveness of the mammary gland is underscored by observations of raised breast cancer risk after increased cumulative dosage of reproductive hormones (by early menarche, late menopause, or progestin-containing hormone replacement therapy; ref. 15). However, so far no direct link between changes in hormone responsiveness and the breast cancer-protective effect of pregnancy at early age could be demonstrated. With respect to sensitization of proapoptotic pathways studies are ongoing using retinoids, which not only cause differentiation, but also enhance the expression of the DNA damage response protein p53 (16). Early pregnancy-induced long-lasting alterations in the ECM or stroma of the breast/mammary gland have been observed in rodents and women (17), but so far have not resulted in the identification of promising pharmaceutical targets. Among all theories, the highest potential for translation into a breast cancer-preventive strategy has been attributed to the hypothesis of a molecular switch in mammary stem/progenitor cells upon early-age pregnancy, rendering them less proliferative and less susceptible to oncogenic transformation (18). Indeed, the longevity and self-renewing properties of stem/progenitor cells make them not only prime targets for malignant cellular transformation and carcinogenesis, but also for the protective effect of early-age pregnancy. In this regard, initial studies investigating the influence of early-age pregnancy on the regenerative potential of mammary cells in mice yielded controversial results. Whereas one study observed an apparent parity-induced decrease in repopulating units (19), a second study found no influence of parity on the number of stem/progenitor cells with regenerative capacity (20). These studies differed in various aspects, including age at mating and cell isolation procedures. Furthermore, because both studies used unfractionated total mammary (epithelial) cells, subtle qualitative alterations of critical stem/progenitor cell properties could have been missed, emphasizing the need for more focused studies with fractionated mammary stem/progenitor cells. Such studies have recently been performed in mice and humans, and revealed remarkable translational consistencies in parity-induced qualitative alterations of mammary stem/progenitor cell fate (Fig. 1; refs. 3, 4).

Early-Age Pregnancy Affects Mammary Stem/Progenitor Cell Fate via Wnt and Tgf β Signaling

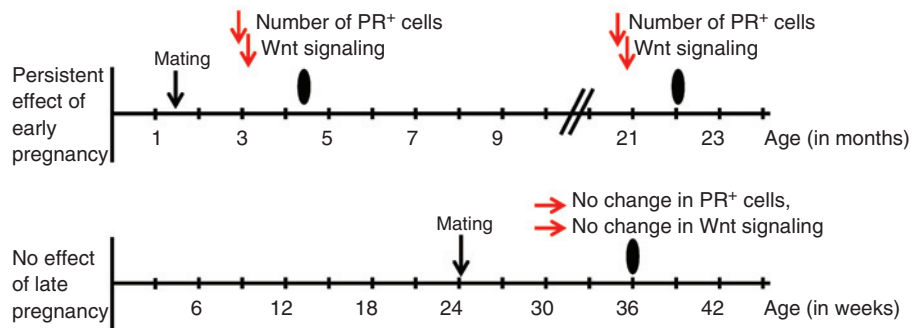
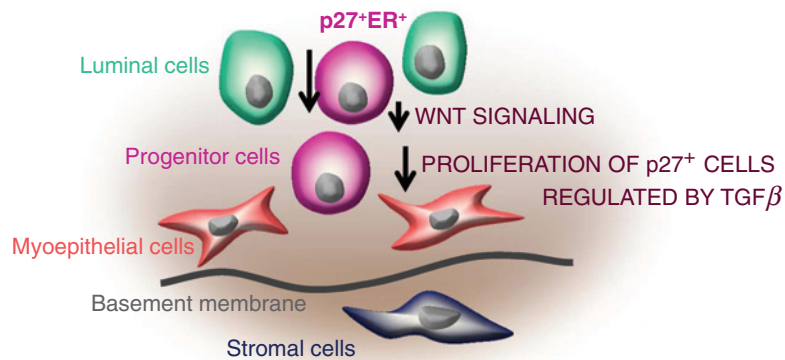
Investigating subfractionated mammary epithelial cell subtypes and thus eliminating background noise from mixed cell

populations, a recent study in mice found decreased activity of stem/progenitor cell-related signaling pathways upon parity (3). In particular, a reduction in cell fate-determining Wnt signaling was observed in mammary stem/progenitor cells after early-age pregnancy in mice (Fig. 1A). This was associated with a decreased proliferation potential of mammary stem/progenitor cells *in vitro* and *in vivo*. Mechanistically, the proliferation block was caused by early parity-induced reduction in hormone receptor-positive (ER⁺ and PR⁺) and Wnt ligand Wnt4-secreting luminal epithelial cells. The causal relationship between decreased luminal Wnt4 expression, reduction of Wnt signaling and associated proliferation failure was substantiated by the finding that recombinant Wnt4 was able to rescue *in vitro* the parity-induced proliferation defects of the isolated mammary stem/progenitor cell subpopulation (3). Given the well-established tumorigenic function of the Wnt pathway (10), the findings indicate a key involvement of Wnt signaling in the breast cancer-protective effect of early-age pregnancy. Interestingly, and most importantly, the early pregnancy-induced reduction in the proportion of ER⁺/PR⁺ luminal cells and in epithelial Wnt signaling persists into advanced age, and thus is of life-long duration in mice (Fig. 1B; ref. 21). Furthermore, pregnancy at a late age had only a marginal or even no effect on hormone receptor-positive cells and epithelial Wnt signaling (Fig. 1B; ref. 21). These data underscore the critical interdependence of the different mammary epithelial cell subpopulations for normal as well as pathologic mammary gland development. Moreover, they are in full accordance with the life-long breast cancer-protective effect of early- but not late-age pregnancy in humans, and validate the adopted mouse model for studying prevention strategies against human breast cancer.

The effects of parity on specific mammary epithelial cell subpopulations have been investigated recently also in humans (4). The studies also found a downregulation of protumorigenic pathways such as Wnt signaling in mammary epithelial progenitor cells isolated from breast tissue of parous women (Fig. 1C). Furthermore, parity in women induced a decrease in the number of mammary hormone-sensing (ER⁺) cells (4, 22), an apparent reduction in Wnt 4 expression (as can be derived from the presented raw data), and a cellular proliferation block in the corresponding breast tissues (4). These similarities between mice and women regarding parity-induced cellular and molecular changes in mammary epithelium strongly indicate that decreased progesterone- and Wnt4-mediated Wnt signaling in mammary stem/progenitor cells plays a key role in the protective effect of early-age pregnancy against breast cancer. This is underscored by the well-established protumorigenic function of Wnt signaling in mammary stem/progenitor cells of mice and humans (10). Hence, its decrease upon pregnancy at early age paves the way for the use of Wnt target expression as possible biomarkers for breast cancer risk and of Wnt inhibitors as potential preventive agents against breast cancer. However, in addition to decreases in cell fate-determining and protumorigenic Wnt signaling in mammary progenitor cells, breast tissue of parous women showed also a decreased frequency and quiescence of progenitor cells expressing high levels of the cell-cycle regulator p27 (encoded by *CDKN1B*; Fig. 1C). A subset of these p27⁺ cells were hormone-responsive (ER⁺) cells and exhibited concomitant high expression of phospho-Smad2 (pSmad2), a key mediator of TGF β signaling, indicating that quiescence of mammary hormone-responsive progenitor cells after parity is regulated by TGF β signaling (4).

A Early pregnancy–induced cellular and molecular changes in mice**Figure 1.**

Early pregnancy–induced cell fate changes in mice and humans. A, in mice, early-age pregnancy reduces the number of hormone-sensing and Wnt4-secreting luminal cells, which leads to a decrease of protumorigenic Wnt signaling in mammary stem/progenitor cells (3). B, early-age pregnancy-induced cellular and molecular changes persist until old age and are not induced by late-age pregnancy in mice. Mice were mated either at 6 weeks of age (early pregnancy) or at 24 weeks of age (late pregnancy). Mammary glands were harvested for analysis either 40 days after weaning or at the old age of 22 months (21). C, in women, early-age pregnancy reduces the number of hormone-sensing p27⁺ (p27⁺ER⁺) progenitor cells and decreases protumorigenic Wnt signaling and proliferation (induction of quiescence) in mammary epithelial progenitor cells (4).

B Duration of changes and effect of age at pregnancy in mice**C Pregnancy-induced cellular and molecular changes in women**

These findings are reminiscent of previous observations in mouse mammary glands showing that TGF β is activated in ER⁺ cells and regulates their quiescence (23). Hence, Wnt signaling may act together with quiescence-inducing TGF β signaling in mediating the breast cancer–protective effect of pregnancy implying that a combination of Wnt inhibitors and TGF β -modulating agents might be required for optimal pharmacologic prevention against breast cancer. Alternatively, rodent data suggest that short-term "vaccination" with high-dose pregnancy hormones can provide life-long protection

against ER⁺/PR⁺ breast cancer in young women (8, 9). Because estrogen and progesterone affect mammary tumors, further studies are needed to eliminate potential harmful effects of such approaches. Overall, although it cannot be excluded that additional cell fate changes are required for full pregnancy-mediated breast cancer protection, the congruent and complementary findings in mouse and human mammary epithelial stem/progenitor cells (Fig. 1) provide promising initial targets for translational studies directed toward the development of biomarkers and breast cancer prevention strategies.

Conclusions and Future Challenges

Similar cellular and molecular effects of early pregnancy in mice and humans include (i) their predominant restriction to mammary epithelial stem/progenitor cells, (ii) reduction of progesterone-sensing and Wnt4-secreting (luminal) epithelial cells (mice) and/or quiescence of hormone-responsive p27⁺ progenitor cells (humans), and (iii) decreased proliferation and protumorigenic potentials through inhibition of Wnt4-dependent Wnt signaling (mice) and/or a combination of Wnt inhibition and TGF β -dependent quiescence of p27⁺ progenitor cells (human; Fig. 1). The robustness and the (life-long) persistence of the findings in mice and the closely similar findings in humans provide a strong basis for further translational studies involving iterative cycles of experimentation in the rodent model and in human breast tissue. These studies must include: (i) investigation of the effects of hormonal treatments mimicking pregnancy at early age on gene expression and proliferation potential of mammary epithelial stem/progenitor cells in mice. This experimental model will be essential for the demonstration of a direct link between decreased Wnt signaling in mammary stem/progenitor cells and pregnancy-mediated breast cancer protection. (ii) Validation of the role of decreased hormone-sensing cells and reduced Wnt signaling in breast tissue of women following early (<20-years-old) versus late (>35-years-old) first pregnancy using appropriate biomarkers such as PR, Wnt4, nuclear β -catenin, versican, keratin15, and p27. (iii) Testing Wnt inhibitors such as tankyrase inhibitors, porcupine inhibitors, and anti-frizzled receptor antibodies in mice and high-risk women for potential breast cancer-preventive effects (24). Because Wnt is downstream of PR, PR antagonists such as Ulipristal, which is already licensed

for use in uterine fibroids, could also be considered. (iv) Screening for epigenetic alterations as a possible cause of the early pregnancy-induced life-long changes of gene expression in mammary epithelial stem/progenitor cells. And (v) more specific characterization of the mammary epithelial stem/progenitor cell types involved in pregnancy-mediated breast cancer protection and their relationship to cancer stem cells in mouse and human breast tissue (25). If these challenges can be met, the lessons learned from the new insights into the mechanisms of early-age pregnancy-induced protection against breast cancer can be of considerable help to counteract the predicted increase in breast cancer-related mortality in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Meier-Abt, C. Rochlitz
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