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COMMENTARY

Postpartum Remodeling, Lactation, and Breast Cancer Risk: Summary of a National Cancer Institute–Sponsored Workshop

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The pregnancy–lactation cycle (PLC) is a period in which the breast is transformed from a less-developed, nonfunctional organ into a mature, milk-producing gland that has evolved to meet the nutritional, developmental, and immune protection needs of the newborn. Cessation of lactation initiates a process whereby the breast reverts to a resting state until the next pregnancy. Changes during this period permanently alter the morphology and molecular characteristics of the breast (molecular histology) and produce important, yet poorly understood, effects on breast cancer risk. To provide a state-of-the-science summary of this topic, the National Cancer Institute invited a multidisciplinary group of experts to participate in a workshop in Rockville, Maryland, on March 2, 2012. Topics discussed included: 1) the epidemiology of the PLC in relation to breast cancer risk, 2) breast milk as a biospecimen for molecular epidemiological and translational research, and 3) use of animal models to gain mechanistic insights into the effects of the PLC on breast carcinogenesis. This report summarizes conclusions of the workshop, proposes avenues for future research on the PLC and its relationship with breast cancer risk, and identifies opportunities to translate this knowledge to improve breast cancer outcomes.

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Beginning with pregnancy, the breast undergoes a cyclical transformation during which it matures from a resting, nonfunctional gland to a milk-producing organ, which then gradually reverts back to quiescence after cessation of lactation. Data suggest that the pregnancy–lactation cycle (PLC) permanently alters the molecular histology of the breast (1,2) and influences breast cancer risk. In contrast with the extensive literature on breastfeeding and improved infant health (3–6), data related to molecular mechanisms and biomarkers linking the PLC to breast carcinogenesis are sparse. Separating the intertwined, dynamic effects of pregnancy, breastfeeding, pregnancy-related interruption of ovulation and postweaning remodeling of the breast is challenging, which suggests that novel research strategies are needed to study the PLC. To summarize scientific knowledge in this area and identify research priorities, the National Cancer Institute conducted a workshop entitled "Postpartum Remodeling, Lactation and Breast Cancer Risk: Towards Improved Risk Assessment and Prevention." The workshop was designed to foster multidisciplinary discussions among attendees, including epidemiologists and other public health researchers, basic and translational scientists, lactation consultants, obstetricians, and pediatricians. This commentary summarizes findings from the workshop, which was held on March 2, 2012, in Rockville, Maryland.

The PLC: Epidemiological Associations With Breast Cancer Risk

The PLC and Overall Breast Cancer Risk

The hypothesis that lactation reduces breast cancer risk has been assessed in many case–control studies and in a limited number of large cohort investigations. Results have been summarized in two meta-analyses, which included approximately 60 individual studies. Bernier et al., who combined case–control studies, found that compared with parous women who never breastfed, women who had breastfed were at reduced risk of breast cancer (odds ratio [OR] = 0.90, 95% confidence interval [CI] = 0.86 to 0.94) (7). Similarly, another meta-analysis found that lactation conferred a marginal reduction in breast cancer risk (8), which was apparent only among women with four or more births and associated long durations of lifetime lactation (Figure 1). Thus, distinguishing the effects of breastfeeding and parity on breast cancer risk is difficult because the strongest effects of breastfeeding are found among multiparous women. In addition, some large cohort studies have not found an association between lactation and breast cancer (9). Overall, epidemiological evidence suggests that lactation-related protection in the general population is marginal and restricted to long lifetime durations of breastfeeding.

The PLC and Breast Cancer Risk Related to Specific Populations and Tumor Types

Breast cancer is an etiologically heterogeneous disease; risk factor associations, including those for breastfeeding, vary with patient and tumor characteristics (10–12). Early onset tumors occur more frequently among black women than among white women (13), and such tumors are often aggressive and difficult to treat (14). Black women have higher rates of estrogen receptor–negative breast cancers, and more specifically, they may be at heightened risk for basal-like cancers (15). In the Carolina Breast Cancer Study, not breastfeeding and elevated waist-to-hip ratio were the

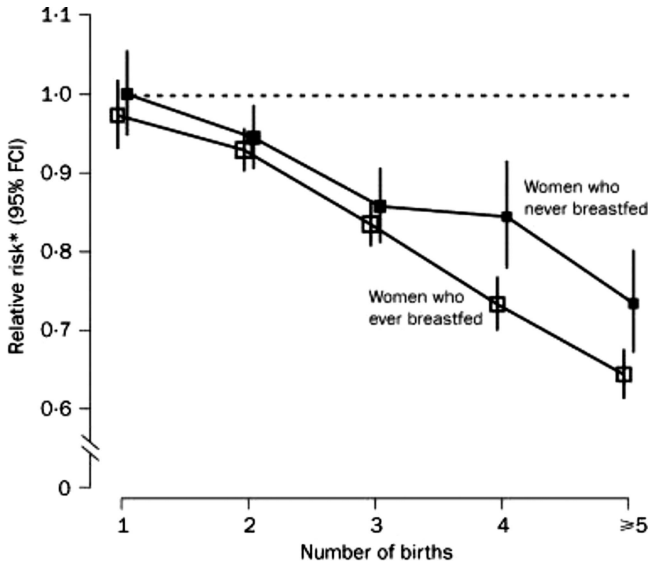

Figure 1. Relative risk of breast cancer in parous women according to breastfeeding history and number of births. Estimates from reanalysis of 47 epidemiological studies (cohort and case–control) conducted in 30 different countries, with parity, lactation, and breast cancer status available for more than 122000 women. Risk estimates diverge based on breastfeeding status at four or more births. Women who ever breastfed and had four children (for a median duration of breastfeeding of 16 months) had a floating absolute risk of 0.73 (floating standard error [FSE] = 0.020), whereas women who had four children but never breastfed had a floating absolute risk of 0.84 (FSE = 0.038). At five births, floating absolute risk was 0.73 (FSE = 0.039) for parous women who did not breastfeed and 0.64 (FSE = 0.020) for women who breastfed a median duration of 30 months. *Relative risk was calculated as floating absolute risk and stratified by study, age, age at first birth, and menopausal status. FCI = floating confidence interval. Permission for reproduction from (8).

strongest risk factors for basal-like breast cancers, with an attributable fraction of 68% among premenopausal black women (15). Not breastfeeding was also linked to increased risk of estrogen receptor–negative/progesterone receptor–negative breast cancers in the Black Women's Health Study (16) and to elevated risk of triple-negative tumors (which include many basal-like cancers) in a largely white population (17). In the Women's Health Initiative study, breastfeeding was not significantly related to risk of estrogen receptor positive or triple-negative cancers, based on 176 cases of the latter (18).

Women who are carriers of *BRCA1* mutations are at increased risk of early onset breast cancers, particularly of the basal subtype. Kotsopoulos et al. reported that *BRCA1* carriers who breastfed for more than 1 year had a 32% reduction in breast cancer risk (OR = 0.68, 95% CI = 0.52 to 0.91), with a potentially greater effect among those who breastfed for more than 2 years (OR = 0.51, 95% CI = 0.35 to 0.74) (19). Breastfeeding was protective for both early- and late-onset cancers. In contrast, breastfeeding was not statistically significantly protective among *BRCA2* mutation carriers, a group that is predisposed to develop molecular subtypes of breast cancers similar to those found in the general population (ie, mainly estrogen receptor–positive).

In another analysis, breastfeeding was modestly protective for breast cancer among premenopausal women, but it was highly protective among those with a first-degree relative with breast cancer (OR = 0.41, 95% CI = 0.22 to 0.75) (20). Risk was unrelated to total duration of breastfeeding, exclusive breastfeeding, or lactation-associated amenorrhea. In this same report, women who never breastfed and used lactation suppressants were at lower risk.

Terminal duct lobular units (TDLUs) are the functional structures of the breast and the site at which most breast cancers arise. Data suggest that the PLC affects the morphology of TDLUs, as observed later in life. Specifically, an analysis of tissues donated by healthy nonpregnant women found that breasts of nulliparous women contain fewer TDLUs per unit area than those of parous women (2). However, individual TDLUs of parous women show greater involution (ie, loss of acini and reduced size) compared with those of nulliparous women (2). Greater levels of TDLU involution have been linked to reduced breast cancer risk (21), and lack of involution may be particularly associated with basal-like breast cancers (22). Thus, one mechanism by which long-term breastfeeding could reduce risk of breast cancer, especially basal-like breast cancers, is by promotion of patterns of TDLU involution that are protective. However, this hypothesis is difficult to test because it requires access to breast tissues donated by women who breastfed for lengthy periods. Understanding effects of breastfeeding on breast cancer risk also adds complexity to addressing other issues in breast cancer epidemiology, including whether rates increase following pregnancy [a controversy reviewed in (23,24)], why early onset tumors may behave aggressively (14), and why some exposures may produce opposite effects on risk at different ages (25).

The PLC and Hormones Related to Breast Cancer Risk

Among premenopausal women, elevated circulating testosterone concentrations are associated with increased breast cancer risk (26); however, clear associations between premenopausal levels of other androgens or estrogens and breast cancer are lacking [reviewed in (27)]. Among postmenopausal women, higher circulating levels of several estrogens and androgens are associated with progressively increased breast cancer risk, independent of other factors, with strongest effects for free estradiol (relative risk = 2.58, 95% CI = 1.76 to 3.78, comparing highest with lowest quintile) (28). Limited prospective data also suggest that higher prolactin levels are associated with a slight increase in breast cancer risk, irrespective of menopausal status (29).

During pregnancy, blood levels of estrogens and progesterone rise linearly, reaching peak concentrations far higher than those observed among nonpregnant women (30). Levels of these hormones vary with multiple factors, such as parity, maternal age, smoking, and fetal gender (30,31). After delivery, circulating estrogen and progesterone levels fall dramatically. Among both pre- and postmenopausal women, pregnancy history is associated with subsequent lower prolactin levels (32,33), whereas neither breastfeeding nor increasing number of children is associated with prolactin levels later in life (32,34). Estrogens and androgens in postmenopausal women do not appear to be strongly related to the PLC (35); few studies have examined these associations in premenopausal women. Women with high levels of prolactin and low total estradiol during late pregnancy tend to have lengthier periods of postpartum amenorrhea (36). High androgen levels during pregnancy have also been associated with shorter breastfeeding duration (37).

The PLC and Breast Cancer Risk: Conclusions and Future Directions

Evidence suggests that breastfeeding is modestly protective for breast cancer overall but may be substantially protective for basal-like breast cancers. Thus, data on breastfeeding provide further evidence for etiologic heterogeneity in breast cancer. In addition, this interpretation suggests that increasing breastfeeding may reduce the incidence of aggressive subtypes, and thus mortality, especially among black women, a group that lags in progress toward meeting breastfeeding goals outlined in the Healthy People 2020 initiative (6).

Conducting epidemiological studies of the PLC is inherently difficult. Women diagnosed with breast cancer may report breastfeeding history differently than healthy women. Reasons for not breastfeeding may bias analyses if these factors are not considered. There are many reasons why mothers do not breastfeed (lactate) or do not breastfeed exclusively, including personal choice, lack of support or information about breastfeeding/lactation, and health issues affecting the mother or infant [reviewed in (4,38,39) and related references]. Women living in wealthier nations, especially if employed outside the home, are more likely to breastfeed for a short period after birth and/or pump milk at convenient intervals rather than to breastfeed in response to infant demands (40). It is unknown whether the timing or frequency of breastfeeding is associated with breast cancer risk. Additional knowledge about breastfeeding practices that most strongly reduce breast cancer risk would aid personal and public health decision making. Whether women breastfeed or not, the breast undergoes dramatic alterations during pregnancy that prepare it for future lactation, and these changes are difficult to separate from postpartum events.

Among parous women, increasing numbers of births are associated with reduced risk of estrogen receptor–positive breast cancer, the most common tumor subtype (10–12). Multiparity is also related to greater attained age and lifetime duration of lactation, which makes separating these effects difficult. Furthermore, early age at first birth, an established protective factor for breast cancer (41), may be related to multiparity, creating additional analytical complexity. For these reasons, an analysis of women following a first singleton pregnancy may offer advantages, but older primiparous women will include a subset of women who have used infertility treatments and therefore may have a distinctive set of breast cancer risk factors.

The Nurses' Health Study II has developed a questionnaire module to assess lactation history in detail by pregnancy, and the Black Women's Health Study has implemented an online module for pregnancy history and lactation. If feasible given priorities, questionnaires should collect data by pregnancy, including duration of exclusive and total breastfeeding, reasons for stopping or not starting breastfeeding, and use of lactation suppressive medication.

A fundamental unanswered question is whether the protective effects associated with the PLC reflect indirect systemic effects, such as lowering blood levels of circulating hormones, direct effects that occur in the nursed breast, as previously suggested (42), or both. In addition, the high hormone levels during pregnancy could increase breast cancer risk in the short term, especially for some molecular subtypes, whereas the long-term protective effects of pregnancy might reflect different mechanisms, such as greater TDLU involution.

Apart from sex-steroid hormones, few circulating factors have been assessed in relation to time since delivery, and most available data are from postmenopausal women. In considering how the PLC might affect breast cancer risk through modulation of hormones or growth factor levels, it will be important to account for the time of the measurement, changes over time, and cumulative exposure. In addition, improved methods for measuring hormones and other circulating factors among young women and for assessing gene expression in the breast are needed.

Milk as a Biospecimen for Studying the PLC

Noncellular Components in Breast Milk

Milk is rich in growth factors, chemokines, and immunomodulatory, anti-inflammatory and proinflammatory mediators, which are variably derived from leukocytes and breast epithelial cells (43–48). Several of these factors have suggested roles in breast development and/or breast cancer, including interleukins, tumor necrosis factor alpha, epidermal growth factor, and others. Many of these markers show substantial interindividual differences and temporal patterns related to parturition and the health status of the mother–infant dyad. Dvorak et al. found that epidermal growth factor levels are remarkably elevated in milk after extremely preterm deliveries (23–27 weeks) (45), and limited data suggest that these mothers are at elevated breast cancer risk (49,50), which is of interest given the critical role of epidermal growth factor in breast cancer, including the basal subtype (51).

Milk contains oligosaccharides and glycosylated proteins that are generally stable and can be assessed using mass spectrometry (52). Glycosylation can alter the molecular function of some proteins, which may have implications for breast carcinogenesis. Ongoing studies seek to determine whether specific glycosylation patterns are predictive of diseases (eg, diabetes), some of which are related to cancer risk. Recent technological advances have made it possible to profile glycosylation patterns in large sample sets (53–56). For example, mass spectroscopy can be applied to analyze glycosylation patterns in dried milk spots, thus allowing flexible sample collection approaches (57).

Cellular Components in Breast Milk

Milk contains mature epithelial cells, leukocytes, and potentially putative stem-like or progenitor cells (58,59). Therefore, breast milk can serve as a noninvasive source for studying cells of the mature gland. In an analysis of milk donated by 102 women, Wong et al. found that milk contained, on average, approximately 2×10^5 cells/mL, although counts ranged widely and were largely independent of mothers' and infants' ages and mothers' histories of prior pregnancies (60). These researchers also isolated an epithelial cell-enriched fraction, which contained approximately 3×10^4 cells/mL, resulting in approximately 2.5 μg of DNA per donation, which is a favorable yield compared with nipple aspiration and ductal lavage.

Thompson et al. (61) and Ambrosone et al. (62) expanded milk research by analyzing DNA adducts in milk. Recently, Arcaro et al.

have used epithelial cell-enriched milk fractions to detect DNA methylation in tumor suppressor genes by pyrosequencing (60,63). Additional research is needed to assess whether DNA methylation of tumor suppressor genes in milk is associated with breast cancer risk. Toward that end, preliminary comparisons show that levels of DNA methylation are similar in milk from the left and right breasts of women who have had a benign biopsy, whereas among women with cancer, levels are higher in the affected breast. (64).

Optimization and standardization of the timing of milk collection for research with regard to date of delivery, time of day, and breastfeeding is needed to advance the use of this specimen. Milk may be expressed by hand or by pumping with devices that vary in suction strength and the speed with which they empty the breast (65). Data suggest that these methods collect similar volumes of milk but composition may vary. Women whose breasts produce and store greater volumes of milk secrete milk of more variable content, potentially reflecting the influence of incomplete emptying between feeds; furthermore, a woman's milk production may be asymmetric (66). Milk composition, milk fat, and cell content may vary not only within the first year of feeding but also within a single feeding, with higher fat and cell content in the "hindmilk" expelled at the end of feeding, as opposed to the initially expelled "foremilk" (67). A more recent study showed that changes in the milk fat and cell content continue after the end of feeding, peaking within 30 minutes following a substantial volume feed (68). Therefore, diurnal variation in milk fat and cell contents seems to be strongly associated with the degree of fullness of the breast. Given that patterns of breastfeeding affect both milk production and composition, they represent a consideration in measuring biomarkers (65).

Milk as a Biospecimen for Studying the PLC: Conclusions and Future Directions

Future research could leverage analysis of breast milk as an important plentiful, noninvasive, and easily accessed source of various cell populations within the lactating breast. Improved techniques for isolating both epithelial and nonepithelial cells from milk are needed, and progress may occur in tandem with development of better methods of preserving milk because cell fragility may be limiting. Freezing and thawing fresh milk leads to cell lysis, which limits analysis of epithelial cell-enriched fractions, whereas shipping chilled liquid milk is complicated and expensive. Furthermore, optimal methods for temporarily storing milk before processing are needed. Conducting in-person milk collections for research is challenging, especially where postdelivery care is decentralized. Improved methods of preserving cells in milk could enable more flexible means of shipping to laboratories, and improved fractionation would increase sensitivity for detecting cancer-related markers and mechanistic studies. Improved preparation may also facilitate a wide range of cytological analyses using immunohistochemistry, in situ hybridization, flow cytometry, and related methods that can potentially identify rare events with cellular localization, which may provide biological insights about the PLC and breast cancer.

The ability to analyze soluble macromolecules should advance quickly as technologies improve. A critical challenge for this research is identifying normative patterns of change with time since birth, given that collecting milk donations in narrowly defined intervals is impractical. Patterns of change over time may be highly informative. Methods for ensuring that factors influencing milk volume are not drivers of measured concentrations are needed to optimize the utility of these assays.

Animal Models and Mechanistic Studies of the PLC

In murine models, activation of an involution gene signature occurs in the mammary gland within a few hours of weaning. Various patterns of gene expression are observed, with some genes being maximally expressed within 12 hours and others gradually rising during the first 4 days of involution in the mouse. However, with the exception of dying cells that detach into the alveolar (acinar) lumen, morphological changes are not observed until 48 hours of involution. *Stat3* has emerged as a key mediator of the involution process in the mouse mammary gland (69–73). Involution proceeds in two phases: 1) a potentially reversible phase, lasting until approximately 48 hours postweaning in mice, and 2) an irreversible phase beginning in mice at 48 hours postweaning that results in tissue remodeling (Figure 2). In mice, most of the alveolar epithelium is removed by 6 days postweaning, and prepregnancy gland morphology is reestablished by 10 days of involution, albeit with subtle changes to branching morphology and altered gene expression profiles. *Stat3* activation mediates involution by induction of cell death in alveolar epithelium and by recruitment and alternative activation of macrophages (70). Mammary glands from conditionally deleted *Stat3* mice demonstrate a "failed involution" phenotype marked by reduced cell death and inflammatory signaling, delayed remodeling, and a prolonged reversible phase (74).

In animals, postweaning remodeling of the mammary gland is a proinflammatory process, which has led to the proposal of the "involution hypothesis" (75–84). This ascribes a tumor- and metastasis-promoting character to the postpartum microenvironment. In support of this hypothesis, when human mammary tumor MCF10DCIS cells are injected into the mouse mammary gland 1 day postweaning, tumor size and lung infiltration of the cells were statistically significantly greater than in nulliparous controls (75). Characterization of the involuting mouse mammary gland suggests that increased collagen deposition, occurring specifically during postpartum gland involution, leads to upregulation of COX-2 and enhanced migration of tumor cells (75). Injecting ibuprofen (which inhibits COX-2 signaling) concurrent with tumor cells at 1 day postweaning reduced collagen deposition and tumor growth to levels similar to that in nulliparous controls, which suggests the importance of a collagen and COX-2 proinflammatory pathway in the promotion of tumor aggressiveness in the involuting mammary gland (75,84) (Figure 3). Importantly, macrophage infiltration and collagen deposition have been reported in human breasts undergoing postpartum involution.

Data pointing to involution-associated inflammation as a factor in the development of aggressive breast cancer is also consistent with observational data from humans. Among women aged less than 40 years who develop breast cancer, survival is worse if the breast cancer is diagnosed between 4 to 7 months postpartum compared with diagnosis during pregnancy or at other times outside of this interval (85–87). This poorer prognosis may extend

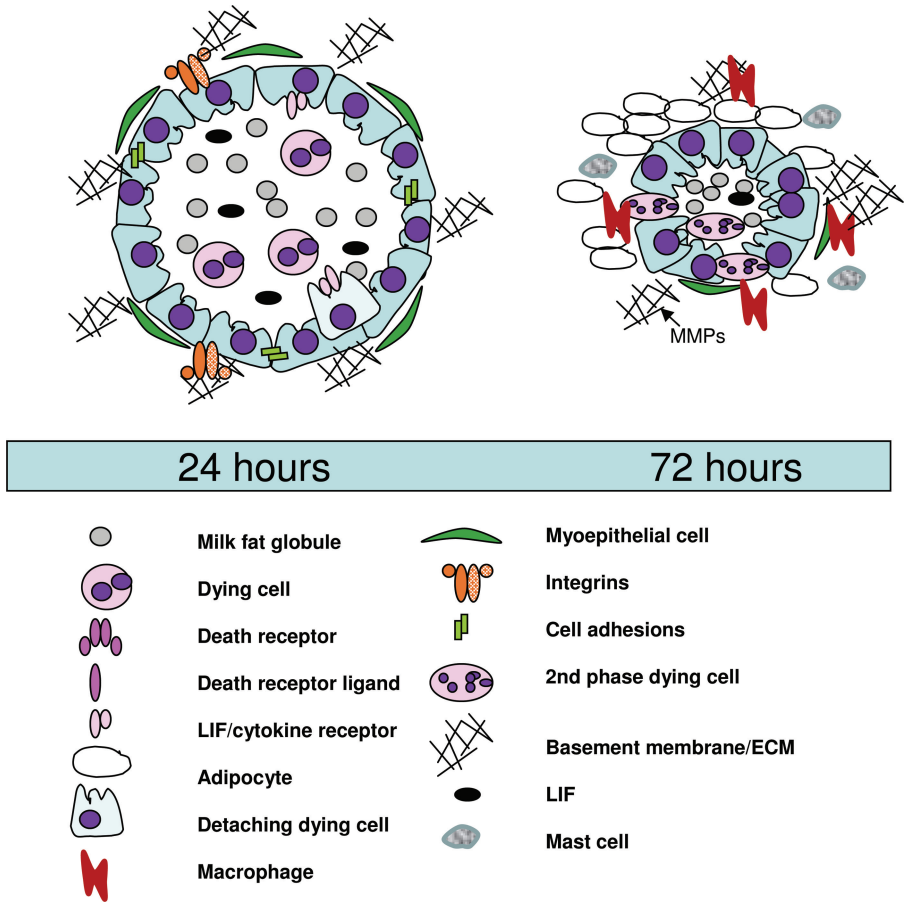

Figure 2. Schematic representation of the involution process. Involution occurs in two phases. The first phase is reversible phase, lasting up to 48 hours in the mouse, that is characterized by extensive cell death, which results in detachment of dying cells into the alveolar lumen. These cells upregulate cell surface markers such as CD14 in a Stat3-dependent manner and are subsequently phagocytosed by the viable epithelium. In the second phase of involution, tissue remodeling and redifferentiation of adipocytes is associated with a second wave of cell death that returns the gland to a near-prepregnant state. The second phase is associated with an influx of macrophages and mast cells that facilitate tissue remodeling and with extracellular matrix (ECM) breakdown by matrix metalloproteases (MMP). LIF = leukemia inhibitory factor. Adapted from (73).

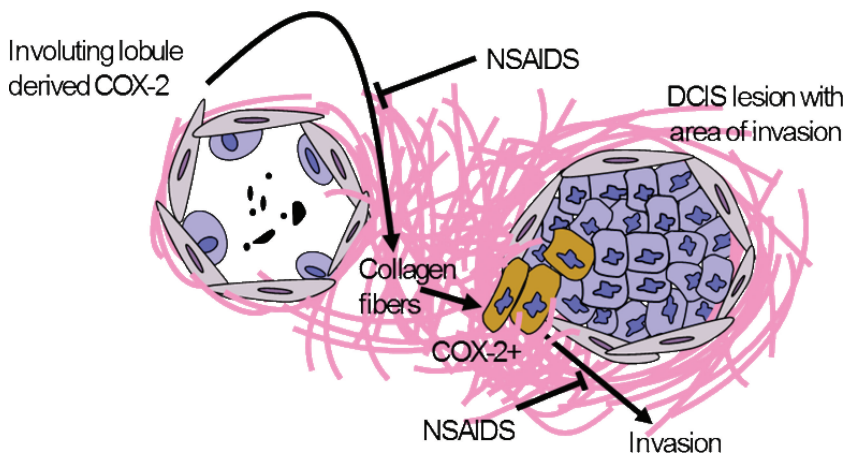

Figure 3. Proinflammatory involution environment promoting breast carcinogenesis. A model depicting COX-2, derived from the involuting mammary gland, mediating collagen fibrillogenesis and COX-2 promoting invasion (brown cells) of tumor cells exposed to involution collagen. NSAIDS = Nonsteroidal anti-inflammatory drugs; DCIS = Ductal carcinoma in situ. Reproduced with permission from (75).

to breast cancers diagnosed within 5 years of pregnancy (88). Data also suggest that use of aspirin or nonsteroidal anti-inflammatory drugs may improve breast cancer survival [(89) and reviewed therein].

Animal Models and Mechanistic Studies of the PLC: Conclusions and Future Directions

Animal models may improve our knowledge of the mechanisms by which the PLC may influence breast cancer risk. A major

Box 1. Summary and research recommendations from the National Cancer Institute workshop "Postpartum Breast Remodeling, Lactation, and Breast Cancer Risk: Towards Improved Risk Assessment and Prevention"

Summary of Research Presented

1. In the general population, long durations of lactation are associated with a small reduction in overall breast cancer risk, in addition to many other health benefits for mother and child.
2. Breastfeeding may provide greater protection against triple-negative, basal-like, and BRCA1 mutation–associated breast cancer, suggesting particular protection against aggressive types of tumors.
3. In humans, effects of parity and lactation on breast cancer risk are largely inseparable.
4. First pregnancy induces hormonal changes, some of which persist long-term. It is unclear how lactation affects the long-term hormonal environment over and above that of pregnancy.
5. Data from animal models demonstrate a molecular "involution signature" regulated by Stat3 signaling.
6. Recent pregnancy may increase near-term risk of breast cancer and poor prognosis. In the rodent model, involution of the mammary gland demonstrates shared characteristics with wound healing and promotes tumorigenesis. Nonsteroidal anti-inflammatory drugs can inhibit tumorigenesis induced by involution in this model.
7. Aspects of the research in rodent models may be applicable to human breast remodeling postweaning; however, the rodent mammary gland differs in composition and organization from the human breast. The degree to which involution induces inflammation may also vary across animal models and from animal to human but may be modifiable.
8. Multiple components of milk can be assessed and vary with both physiological and pathological states (eg, weaning, inflammation, infection). These include hormones, cytokines, glycoproteins, and methylation of epithelial cells found in milk. Association of these markers with breast cancer risk is yet to be determined.

Recommendations for Future Research

1. Examine datasets to determine associations between parity, lactation, and breast cancer risk and outcomes, including studies of women who are *BRCA1* mutation carriers and assessment of relationships by molecular subtype.
2. Refine analyses of lactation by collecting more detailed data (eg, duration per child, period of exclusive breastfeeding, weaning strategy/duration).
3. Use animal models to try to separate effects of parity and lactation.
4. Use animal models to examine the role of involution in promoting different breast cancer subtypes and the mechanisms and markers related to these processes.
5. Determine association of parity and lactation with long-term physiological states (eg, hormones, breast stem cells, breast microenvironment).
6. Conduct methods studies that explore collection, storage, and variability (within a woman over time and between women) of milk samples with regard to different markers in milk (eg, hormones, glycoproteins, cytokines, epithelial cells).
7. Examine associations of soluble or cellular components in milk with markers of breast cancer risk (eg, mammographic density).

challenge, however, is making findings from animal studies relevant to human biology. Weaning in animal models is generally a rapid process, whether physiologically or experimentally induced (eg, teat sealing or removal of pups from mother). However, a prolonged weaning model may more accurately reflect the more gradual weaning process that occurs in women. The timing of weaning (prolonged vs rapid) may influence the duration of involution, and, therefore, affect the gene expression program and components of the microenvironment. One report described an expansion of murine mammary stem cells during pregnancy and then a drop to or below baseline levels after weaning (90). Similar rules may apply to the human breast; however, the scarcity of human breast tissue specimens during the PLC poses considerable challenges to conducting such studies in humans. To this end, human breast milk may provide a source of cells for study.

Conclusions

The PLC is a complex period that permanently alters breast biology and influences breast cancer risk. The major research conclusions and future directions emerging through discussions among the multidisciplinary attendees at the National Cancer Institute workshop entitled "Postpartum Remodeling, Lactation and Breast Cancer Risk: Towards Improved Risk Assessment and Prevention" are summarized in Box 1.

The relationship of breastfeeding to breast cancer risk, particularly with regard to specific tumor subtypes, requires further study. Human studies suggest that pregnancy/lactation may offer strong protection against certain types of breast cancer in the long term but may be associated with increased breast cancer risk for other types in the short term. The latter is in agreement with studies in animal models that demonstrate that breast remodeling in the postweaning period may be associated with breast cancer growth and metastases. The protective effects of longer breastfeeding durations against aggressive breast cancer are now supported by a number of studies, particularly for *BRCA1* mutation carriers, but our understanding of the underlying mechanisms is limited. Improving knowledge of PLC effects on the breast stem and/progenitor cell pools and the breast microenvironment in relation to breast cancer subtypes is a priority. Identification of mechanisms and markers related to postpartum breast changes among humans may have value for risk assessment and prevention. As animal research progresses, human studies could be designed to understand breast involution in relation to cancer risk and to explore the importance of inflammatory pathways in driving tumor aggressiveness. Human breast milk may provide a biospecimen for understanding the mechanisms that are important in the PLC and identifying biomarkers with potential value for risk assessment or early breast cancer detection. Given the importance of the PLC in breast carcinogenesis, research in this

area may lead to important translational advances in risk assessment, early detection, or prevention of breast cancer.

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