

# Breast Differentiation and Its Implication in Cancer Prevention

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## ABSTRACT

Sporadic breast cancer is a fatal disease most frequently diagnosed in American women from all ethnic groups, suggesting that primary prevention should be the ultimate goal for breast cancer control. We have developed a novel paradigm for breast cancer prevention arising from the well-established knowledge that an early first full-term pregnancy protects the breast against neoplastic transformation, as well as from our studies of the biological principle underlying this protection. We have shown experimentally that the first pregnancy induces the expression of a specific genomic signature in the breast that results from the completion of a cycle in this organ's differentiation driven by the reproductive process. This signature, in turn, is a biomarker associated with a possible overall lifetime decrease in breast cancer risk. We have shown in an experimental model that a short treatment with human chorionic gonadotropin, a placental hormone secreted during pregnancy, induces the same genomic signature that occurs in pregnancy, inhibiting not only the initiation but also the progression of mammary carcinomas, and stopping the development of early lesions such as intraductal proliferations and carcinoma *in situ*. These observations indicate that human chorionic gonadotropin given for a very short period, only until this genomic signature is acquired, has significant potential as a chemopreventive agent, protecting the normal cell from becoming malignant. This is a novel concept which challenges the current knowledge that a chemopreventive agent needs to be given for a long period of time to suppress a metabolic pathway or abrogate the function of an organ.

## INTRODUCTION

The development of strategies for breast cancer prevention is hindered by the multistep nature of the process (1, 2). Current strategies to prevent breast cancer capitalizes on its estrogen dependence, which can be manipulated to control growth or prevent tumor development utilizing either selective estrogen

receptor modulators (2–4) or aromatase inhibitors (5). However, the inability to predict who will develop breast cancer has required the implementation of broad, population-based strategies utilizing preventive measures that have significant adverse effects and require protracted treatment. These drawbacks have limited the acceptance of these strategies, particularly because most women treated would not ultimately develop breast cancer even if no prophylaxis was given (6). Therefore, what is needed is to precisely identify those women who should take a preventive agent, sparing others who will not develop the disease during their lifetime. It is for this very high-risk subgroup that we have developed a new paradigm for breast cancer prevention.

## BASIS FOR A NEW PARADIGM IN BREAST CANCER PREVENTION

Our paradigm has emerged from epidemiologic observations of a direct association of breast cancer risk with nulliparity and of protection conferred by an early first full-term pregnancy (7–9). We have chosen this specific strategy because it represents a window for learning how a physiologic event produces lifetime protection from breast cancer in a significant percentage of women. Although this physiologic event does not explain all the questions about this complex disease, it provides a blueprint for a new paradigm in breast cancer prevention. This paradigm arises from our studies of the biological principle underlying this protection (10–16). We have shown experimentally that the first pregnancy induces the expression of a specific genomic signature in the breast which results from the completion of a cycle of this organ's differentiation driven by the reproductive process. This signature, in turn, is a biomarker associated with a possible overall lifetime decreased breast cancer risk. More importantly, we have harnessed this biological principle by demonstrating in an experimental model that a short treatment with recombinant human chorionic gonadotropin (hCG), a placental hormone secreted during pregnancy, induces the same genomic signature that occurs in pregnancy, inhibiting not only the initiation but also the progression of mammary carcinomas, stopping the development of early lesions, such as intraductal proliferations and carcinomas *in situ*. These observations indicate that hCG given for a very short period (11, 12) has significant potential as a chemopreventive agent, protecting the normal cell from becoming malignant. This new biological concept also implies that when the genomic signature of protection or refractoriness to carcinogenesis is acquired, the hormonal treatment with hCG is no longer required. This is a novel concept which challenges the current knowledge that a chemopreventive agent needs to be given for a long period to suppress a metabolic pathway or abrogate the function of an organ.

## BREAST DEVELOPMENT AND LOBULAR DIFFERENTIATION

The breast progressively develops from infancy to puberty under the main stimuli of pituitary and ovarian hormones. The

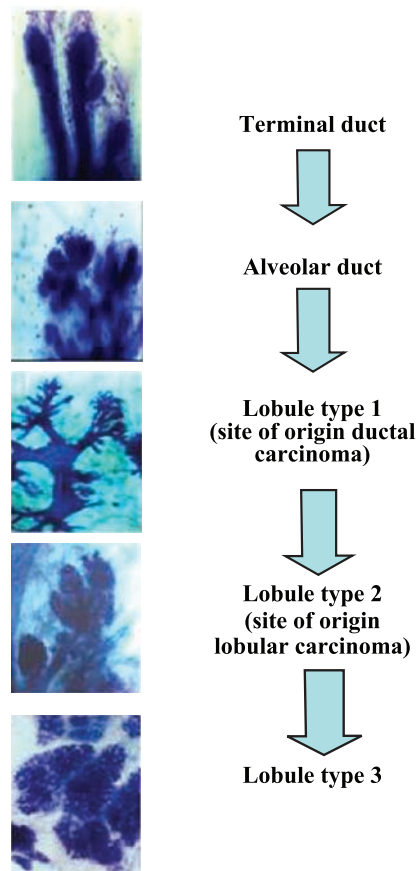
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least differentiated structure identified in the breast of post-pubertal nulliparous women is the lobule type 1 (Lob 1) or terminal ductal lobular unit (TDLU), which progresses to lobule type 2 (Lob 2), which is composed of more numerous ductules per lobule and exhibits a more complex morphology (Fig. 1; ref. 14). During pregnancy, under the stimulus of new endocrine organs, the placenta and the developing fetus, the breast parenchyma branches profusely, leading to the formation of secretory lobular structures. During the first and second trimesters of pregnancy, Lob 1 and Lob 2 rapidly progress to lobule type 3 (Lob 3), which is composed of more numerous and smaller alveoli per lobule (Fig. 1). During the last trimester of pregnancy, active milk secretion supervenes, the alveoli become distended, and the lobules acquire the characteristics of lobule type 4 (Lob 4), when the breast acquires a fully differentiated condition. Lob 4 are present throughout lactation. After weaning, all the secretory units of the breast regress, reverting to Lob 3 and Lob 2 (14). After menopause, all remaining differentiated lobular structures regress, acquiring the appearance



**Fig. 1** At birth, the mammary gland is formed by several excretory ducts, ending in terminal ducts from which elongation and further divisions of the alveolar buds originate. Before the onset of puberty, the ducts and alveolar buds grow and divide in a dichotomous and symphydial basis, forming Lob 1. Lobule formation occurs at menarche. Nulliparous women's breasts primarily contain Lob 1 with some progression to Lob 2, and only minimal formation of Lob 3. Parous women undergo a complete cycle of development through the formation of lobule types 3 and 4 (Lob 4 are not shown), which later regress.

of the Lob 1 of nulliparous women, from which they seem morphologically indistinguishable (14). Nevertheless, the proliferative activity of Lob 1 of nulliparous women at menopause is 2-fold greater than that of the Lob 1 of parous women's breast. The proliferative activity of the mammary epithelium varies as a function of the degree of lobular differentiation, which, in turn, is driven by estrogens and progesterone, as well as by the hormones of pregnancy (14). There is a progressive decrease in the percentage of proliferating cells that react positively to the Ki-67 antibody (Ki-67 or proliferation index) with the progressive maturation of Lob 1 to Lob 2, Lob 3, and Lob 4, differences that are not abrogated when the proliferation index is corrected for the phase of the menstrual cycle (14). Thus, parity, in addition to exerting an important influence in the lobular composition of the breast, profoundly influences the proliferative activity of the parenchyma. The percentage of cells positive for both estrogen receptors and progesterone receptors are highest in Lob 1, and both parameters progressively decrease in an inverse relationship to the degree of lobular differentiation, providing a mechanistic explanation for the higher susceptibility of these structures to transformation by chemical carcinogens *in vitro* (17, 18).

The breasts of nulliparous women almost exclusively contain Lob 1, and their number remains nearly constant throughout the life span of the individual. The breast of early parous women contains predominantly the more differentiated Lob 3, whereas Lob 1 are in a very low percentage until the fourth decade of life; thereafter, their number starts to increase and after menopause they reach the same level observed in nulliparous women (14). The fact that ductal breast cancer originates in Lob 1 (Fig. 1; refs. 17, 18) and the epidemiologic observation that nulliparous women exhibit a higher incidence of breast cancer than parous women (8, 9) indicate that Lob 1 in these two groups of women might be biologically different or might exhibit different susceptibilities to carcinogenesis (14).

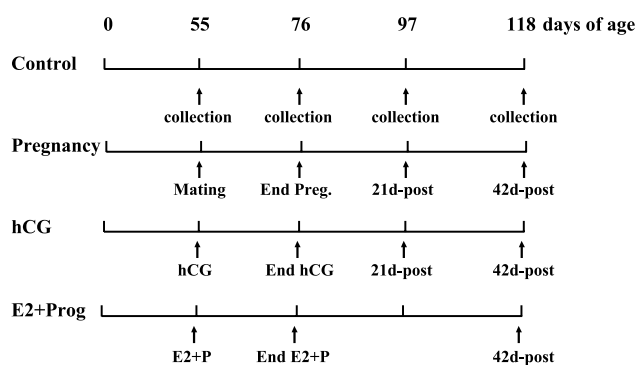
These observations in the human breast have been validated in the rat experimental system in which pregnancy and hCG treatment confers long-lasting protection, indicating that the differentiation induced by these processes is a permanent modification of the biological characteristics of the mammary gland, in spite of the regression of differentiated structures to seemingly more primitive conditions (10–13, 19).

### GENOMIC SIGNATURE INDUCED BY PREGNANCY OR hCG AND SUSCEPTIBILITY OF THE MAMMARY GLAND TO CARCINOGENESIS

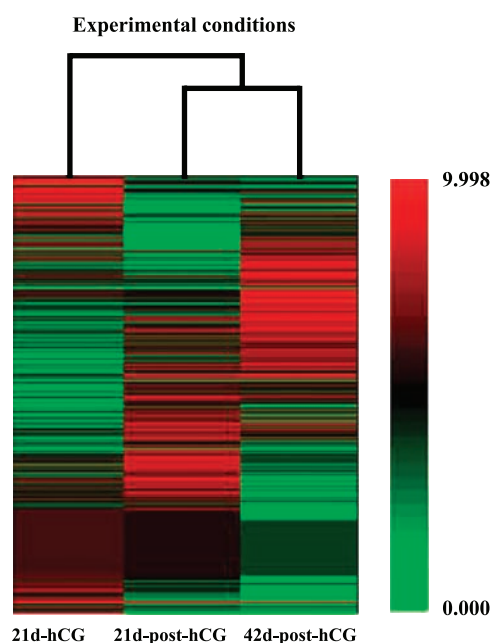
The susceptibility of the mammary gland to being transformed by a chemical carcinogen is modulated by specific biological conditions of the host and of the target organ (20). Tumor incidence and number of tumors per animal is directly proportional to the number of terminal end buds that are at their peak of cell proliferation. Stimulation of the development and differentiation of the gland, resulting in profuse lobular development and depression of DNA synthesis (20), or after completion of a 21-day-treatment of virgin rats with hCG, reduce the susceptibility of the mammary epithelium to transformation by a carcinogen. The reduction in cancer incidence is permanent, as shown by the similar degree of reduction when

7,12-dimethylbenz(a)anthracene is given after a delay of 21, 42, or 63 days after termination of hCG treatment. We have used this model to determine the genomic profile or signature that can explain this process.

The genomic signature was obtained using RNA from mammary glands of rats in their 21st day of pregnancy or hCG treatment, or a combination of 17- $\beta$ -estradiol and progesterone (21), and 21 and 42 days postpartum or treatment, respectively (Fig. 2). RNAs were analyzed for each group of animals and compared with the mRNA of age-matched virgin control rats. RNAs were hybridized to cDNA array membranes that contained 5,800 rat genes (Research Genetics, Huntsville, AL). Hierarchical analysis graphically shows the up-regulated and down-regulated genes at different times of hCG treatment (Fig. 3), and it is clear that the genomic profile varies during treatment. Four clusters of genes were clearly identified, designated A, B, C, and D (Table 1). Cluster A shows genes that were overexpressed (10-fold or more) at 21 days of pregnancy/hCG treatment, but decreased to control values after 21 and 42 days postpartum or posttreatment, respectively. Cluster B was composed of genes that were increased (3-fold or more) at 21 days of pregnancy/treatment and continued rising, reaching the highest peak at 21 days, decreasing by 42 days postpartum/hCG treatment. The up-regulation of catechol-*O*-methyltransferase is significant because it can be involved in the conjugation of estradiol and catechol estrogens, reducing the carcinogenic effect of these hormones. Genes related to the apoptotic pathways were also up-regulated from 3-fold to 5-fold. We have shown that the activation of programmed cell death genes occurred through a p53-dependent process, modulated by *c-myc* and with partial dependence on the bcl2-family of genes (12). In this cluster, inhibins A and B, which are heterodimeric nonsteroidal secreted glycoproteins with tumor suppressor activity, were also included (11). We have found that inhibins are not present in the normal resting mammary gland but are induced by pregnancy or hCG treatment. We have also shown that hCG has an autocrine or paracrine effect on mammary epithelial cells (11). hCG also



**Fig. 2** Experimental protocol to study the effect of pregnancy, hCG, and estrogen and progesterone. The hCG was given daily for 21 days at doses of 100 IU i.p. The estrogen and progesterone were given as a pellet (Innovative Research of America, Sarasota, FL) placed in the interscapular region with a trocar. Each pellet contained 0.72 mg of 17 $\beta$ -estradiol and 200 mg of progesterone. The pellets were left for 21 days and then removed.



**Fig. 3** Expression profile of rat mammary glands. Two-dimensional hierarchical clustering of the data matrix consisting of 5,800 genes (Research Genetics). Rows represent genes and columns represent groups of five animals each treated with hCG and sampled at different times. The gene expression analyses were done using Genesight Software (Biodiscovery, Inc., El Segundo, CA).

activates cluster B of genes in 7,12-dimethylbenz(a)anthracene-induced mammary tumors, indicating that this hormone acts through the same pathways for exerting its preventive and therapeutic effects. Cluster C represents genes whose level of expression progressively increased with time of pregnancy or hCG treatment, reaching their highest levels between 21 and 42 days postpartum or posttreatment (Table 1). G/T mismatch-specific thymine DNA glycosylase gene, which was observed to be up-regulated in Lob 3 of the human breast, was also increased by 5-fold in this model. These data indicate that the activation of genes involved in the DNA repair process is part of the signature induced in the mammary gland by either pregnancy or hCG treatment of virgin animals. These *in vivo* observations confirm our previous findings that the ability of the cells to repair carcinogen-induced damage by unscheduled DNA synthesis and adduct removal is more efficient in the parous and in the hCG-treated virgin than in the untreated virgin animal mammary gland (22). Therefore, a principal mechanism mediating the protection from mammary carcinogenesis conferred by either full-term pregnancy or hCG treatment is the enhancement of the ability of the cells to repair DNA damage, which is in turn the determinant of the lower susceptibility to carcinogenesis. Cluster D genes (Table 1) were up-regulated more than 3-fold at the 15th day of pregnancy or hCG treatment, down-regulated at the 21st day in both pregnant and hCG-treated animals, and remained down-regulated up to 42 days. Cluster D, in combination with cluster C, is a component of the signature induced by hCG in the mammary gland.

Table 1 Genes differentially expressed in the rat mammary gland

Cluster	Genes
A	$\beta$ -casein $\alpha$ -lactalbumin
B	fatty acid-binding protein EST Rn.37635 with high homology to BCL7B gene catechol-O-methyltransferase EST Rn.5953 EST Rn.22912 EST Rn.4339 testosterone repressed prostate message 2 interleukin 1 $\beta$ -converting enzyme bcl2 bcl-XL bcl-XS p53 p21 <i>c-myc</i> inhibin A and B
C	glycogen phosphorylase AMP activated kinase bone morphogenetic protein 4 Vesicle-associated protein 1 G/T mismatch-specific thymine DNA glycosylase
D	pro- $\alpha$ collagen III procollagen II $\alpha$ 1 BTG1 thymosin $\beta$ 4

The hierarchical analysis of the genomic profile 42 days after the treatment with hCG is significantly different from the profile of animals treated with 17- $\beta$ -estradiol and progesterone (Fig. 4). These data show that the genomic signature of the mammary gland induced in virgin animals by exogenous administration of hCG is similar to that induced by pregnancy and different from the signature induced by 17- $\beta$ -estradiol and progesterone. These specific genomic profiles are still manifested 42 days after termination of treatment. The importance of these specific signatures is highlighted by the fact that administration of carcinogen to hCG-treated or control virgin rats whose mammary glands seem morphologically similar will induce a markedly different tumorigenic response, supporting the concept that the differentiation induced by hCG is expressed at the genomic level, and results in a shift of the susceptible stem cell 1 to a refractory stem cell 2 (Fig. 5). The permanence of these changes, in turn, makes them ideal surrogate markers for the evaluation of hCG effect as a breast cancer preventive agent.

### UNIFYING CONCEPTS

Breast cancer originates in the undifferentiated terminal duct of Lob 1 which contains stem cells (stem cell 1; Fig. 5), the site of origin of ductal carcinomas. The susceptibility of Lob 1 to undergo neoplastic transformation has been attributed to its high rate of cell proliferation and of carcinogen binding to the DNA and low reparative activity (19, 22, 23). The hormonal milieu of an early full-term pregnancy or hCG treatment induces lobular development, completing the cycle of differentiation of the breast. This process induces a specific genomic signature in the mammary gland that is represented by stem cell 2 (Fig. 5). Even though differentiation significantly reduces cell prolifera-

tion in the mammary gland, the mammary epithelium remains capable of responding with proliferation to given stimuli, such as a new pregnancy. Stem cell 2 is able to metabolize the carcinogen and repair the induced DNA damage more efficiently than stem cell 1, as has been shown in the rodent experimental system (18, 22). We also have evidence that hCG has an effect in the cancer cell by inducing the differentiation pattern of the mammary gland (12). The finding that differentiation is a powerful inhibitor of cancer initiation provides a strong rationale for identifying the genes that control this process. The basic biological concept is that pregnancy or hCG shifts the stem cell 1 to the stem cell 2 which is refractory to carcinogenesis. The data generated with the new tools provided by cDNA microarrays have shown that although lobular development regressed after the cessation of hormone administration, programmed cell death genes remained activated, but more importantly, a new set of genes (cluster C) reached the maximum expression, whereas another set of genes (cluster D) were down-regulated. Those genes in clusters C and D are the ones providing the genomic signature that is specific for hCG and pregnancy, and are different from the signature induced by other hormones such as estrogen and progesterone.

These mechanisms play a role in the protection exerted by hCG from chemically induced carcinogenesis, and might even be involved in the lifetime reduction in breast cancer risk induced in women by full-term and multiple pregnancies. The implications of these observations are 2-fold: on one hand, they indicate that hCG, like pregnancy, may induce early genomic changes that control the progression of the differentiation pathway, and that these changes are permanently imprinted in the genome, regulating the long-lasting refractoriness to carcinogenesis. The permanence of these changes, in

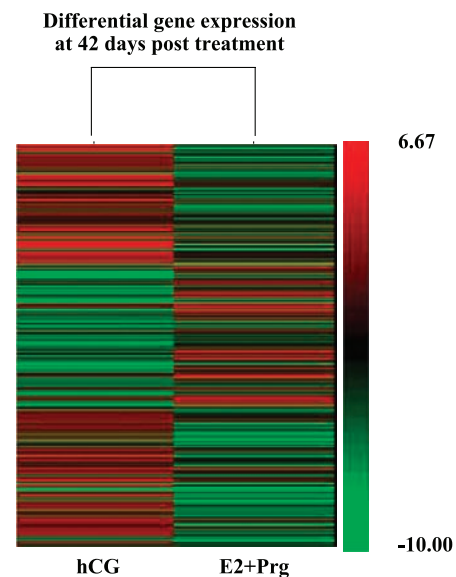


Fig. 4 Expression profile of rat mammary gland. Two-dimensional hierarchical clustering of the data matrix consisting of 5,800 genes (Research Genetics). Rows represent genes and columns represent groups of five animals each treated with hCG and combined estrogen and progesterone pellets sampled at 42 days posttreatment (see Fig. 2). The gene expression analyses were done using Genesight Software (Biodiscovery).

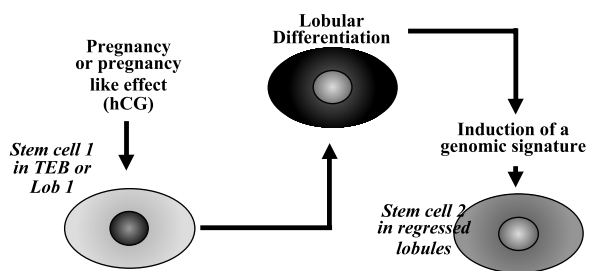


Fig. 5 Pregnancy or hCG treatment for 21 days induces differentiation of mammary gland and shifting of stem cell 1, which is susceptible to carcinogenesis, to stem cell 2, which is refractory to carcinogenesis. Adapted with permission from ref. 19.

turn, would make them ideal surrogate markers of hCG effect in the evaluation of this hormone as a breast cancer–preventive agent.

## OPEN DISCUSSION

**Dr. James Ingle:** The concept of early prevention makes a lot of sense. Are there any programs to explore the feasibility, safety, and ethics of an intervention early in life to simulate pregnancy?

**Dr. Jose Russo:** We are interested in doing that, but we need first to identify which is the genomic signature in the breast tissue. We have a protocol that has been approved to study young premenopausal women with BRCA1 and BRCA2 familial breast cancer in order to treat them for different periods with hCG, and we are now in the first phase of the study. We are using ductal lavage specimens to see what is the genomic signature in these women before and after treatment. We want to compare if the genomic signature is similar to the one that we find in the pregnant women.

**Dr. Angela Brodie:** Some time back, there was a lot of interest in seeing if maybe it was a component of the hCG that was responsible for the protective effect. Has that gone anywhere or do you think it is the actual hCG?

**Dr. Russo:** We entered in an agreement with Serono in Switzerland many years ago because they want to see if the recombinant hCG was different from the hCG obtained from urine. We tested and we demonstrated that there is no difference. It is the hCG by itself.

**Dr. Richard Santen:** If there is a genomic signature for early exposure to pregnancy, one might expect that women with early pregnancy who do develop breast cancer would have breast cancers that would act differently, biologically, from breast cancers in patients who had a late pregnancy. Do we have any data on the long-term follow-up of patients with breast cancer who had early versus late pregnancy?

**Dr. Russo:** We have a 5-year NIH R01 research grant for studying that effect. It is a case-control study of four groups of women: nulliparous women with cancer and without cancer, and parous women with and without cancer. The objective is to answer that question. The reality is, of course, that parous women also develop breast cancer, but we postulate that the genomic signature is different. As a matter of fact, when we studied the development of the breast tissue of women with BRCA1 and BRCA2, we found that the mammary gland

developed differently, meaning that there are genes controlling the way that the mammary gland differentiates in the process that really matters. In a couple of years, we will know the genomic signatures in these populations.

**Dr. Graham Colditz:** When we classify our tumors by ER and PR status, the adverse effect of a first pregnancy is only driving ER-negative, PR-negative tumors, and the protective benefit of parity is predominantly for ER-positive, PR-positive disease and not for the others. So there is a real difference in the adverse effect of first pregnancy and the beneficial effect of additional pregnancies.

**Dr. Santen:** My recollection is that the relative risk of breast cancer with having a first pregnancy before age 25 may be a protective effect of about 0.6. Is that correct?

**Dr. Ingle:** In some of the older studies, pregnancy before age 17 reduces risk by about two-thirds, so there is a direct relationship—the earlier the better.

**Dr. Russo:** If you remember the data from Nagasaki and Hiroshima, the girls who were between 10 and 14 years of age at the time of radiation exposure are the ones who developed a higher incidence of breast cancer, meaning that the pubertal breast is very vulnerable to any carcinogenic exposure. That is the reason why we feel that the developing breast is the target for prevention.

**Dr. Vessela Kristensen:** Have you compared the signatures that you've obtained for this early pregnancy-mimicking event to the signatures of BRCA1 tumors that have been published, and is there anything that indicates that exactly these types of tumors would be successfully targeted by this prevention?

**Dr. Russo:** At the present time, we are working on developing the methodology for studying those women and we don't have those specific data. We are planning to study women who are cancer-free but have a familial history of breast cancer.

## REFERENCES

- Narod S. Prophylactic mastectomy in carriers of BRCA mutations. *N Engl J Med* 2001;345:1498.
- Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. *J Natl Cancer Inst* 2001;93:1633–7.
- Dunn BK, McCaskill-Stevens W, Kramer B, Ford LG. Chemoprevention of breast cancer: The NSABP's breast cancer prevention trial. *J Women's Cancer* 2000;2:177–91.
- Baum M. The ATAC (Arimidex Tamoxifen Alone or in Combination) adjuvant breast cancer trial in post-menopausal women. *Breast Cancer Res Treat* 2001;69:210.
- Mouridsen H, Gershanovich M, Sun Y, et al. Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the International Letrozole Breast Cancer Group. *J Clin Oncol* 2001;19:2596–606.
- Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst* 1989;81:1879–86.
- MacMahon B, Cole P, Lin TM. Age at first birth and breast cancer risk. *Bull World Health Organ* 1970;43:209.
- Lambe MC-C, Hsieh H-W, Chan A, Ekblom D, Trichopoulos H, Adami O. Parity age at first and last birth and risk of breast cancer: A population-based study in Sweden. *Breast Cancer Res Treat* 1996;38:305–11.
- Vessey MD, McPherson K, Roberts MM, Neil A, Jones L. Fertility and the risk of breast cancer. *Br J Cancer* 1985;52:625–8.
- Russo J, Russo IH. Biological and molecular bases of mammary carcinogenesis. *Lab Invest* 1987;57:112–37.

11. Russo IH, Russo J. Role of hCG and inhibin in breast cancer. *Int J Oncol* 1994;4:297–306.
12. Russo J, Russo IH. Human chorionic gonadotropin in breast cancer prevention. In: *Endocrine Oncology*. Ethier SP, editor. Totowa (NJ): Humana Press Inc.; 2000. p. 121–36.
13. Russo J, Janssens J, Russo IH. Recombinant human chorionic gonadotropin (r-hCG) significantly reduces primary tumor cell proliferation in patients with breast cancer. *Breast Cancer Res Treat* 2000;64:161A.
14. Russo J, Hu YF, Silva IDCG, Russo IH. Cancer risk related to mammary gland structure and development. *J Electron Microscop Tech* 1992;52:204–23.
15. Bhat KP, Pezzuto JM. Natural modulators of estrogen biosynthesis and function as chemopreventive agents. *Arch Pharm Res* 2001;24: 473–84.
16. Peters MV. The effect of pregnancy. Prognostic factors, In: *Breast Cancer*. Forrest APM, Kunkler PB, editors. Baltimore: Lippincott Williams & Wilkins; 1968.
17. Russo J, Calaf G, Russo IH. A critical approach to the malignant transformation of human breast epithelial cells. *CRC Crit Rev Oncog* 1993;4:403–17.
18. Russo J, Gusterson BA, Rogers AE, Russo IH, Welling SR, Van Zwieten MJ. Comparative study of human and rat mammary tumorigenesis. *Lab Invest* 1990;62:244–78.
19. Russo J, Russo IH. Role of differentiation in the pathogenesis and prevention of breast cancer. *Endocr Relat Cancer* 1997;4:7–21.
20. Russo IH, Russo J. Mammary gland neoplasia in long-term rodent studies. *Environ Health Perspect* 1996;104:938–67.
21. Mailo D, Russo J, Sheriff F, et al. Genomic signature induced by differentiation in the rat mammary gland. *Proc AACR* 2002;43:5368A.
22. Russo J, Tay LK, Russo IH. Differentiation of the mammary gland and susceptibility to carcinogenesis: a review. *Breast Cancer Res Treat* 1982;2:5–73.
23. Russo J, Tahin Q, Lareef HM, Hu YF, Russo IH. Neoplastic transformation of human breast epithelial cells by estrogens and chemical carcinogens. *Environ Mol Mutagen* 2002;39:254–63.