Journal of Cellular Biochemistry, Supplement 22:58–64 (1995)

Hormonally Induced Differentiation: A Novel Approach to Breast Cancer Prevention

José Russo, MD and Irma H. Russo, MD

Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, PA 19111

Abstract Breast cancer, one of the most common neoplasms in women, develops more frequently in those who are nulliparous or late parous, who experience early menarche and late menopause and have a family history of breast cancer. Early parity, late menarche, early menopause, and hormone deprivation exert a protective effect. The mechanisms modulating these variations in malignancy susceptibility are not known. Epidemiologic and experimental studies indicate that malignancies develop in the mammary gland as a result of exposure to carcinogenic stimuli (*i.e.*, chemical carcinogens, radiation). Neoplastic transformation requires that the gland be under specific developmental and age-related conditions at the time of exposure to such agents. In the rat, maximal susceptibility to neoplastic transformation is exhibited by the highly proliferating and undifferentiated gland of the young, virgin, intact females, whereas the fully differentiated gland of parous rats and virgin rats treated with the placental hormone human chorionic gonadotropin is protected from tumor development. Hormonally induced differentiation of the mammary gland is a novel approach to breast cancer prevention and therapy. The development of clinical protocols capitalizing on the protective effect of hormonal treatments mimicking pregnancy in humans is required to validate observations in experimental animal models, and to determine how they relate to epidemiologic and clinical findings. The feasibility of this approach is supported by the observed parallelism between humans and experimental models in both the site of cancer origin and the changes in breast development occurring with parity. Breast cancer initiates in terminal ductal lobular units or lobules type 1, the most undifferentiated structures frequently found in the breast of young nulliparous women. Lobules type 1 differentiate into lobules type 2, and these into type 3, which progressively develop more alveoli. With pregnancy, these enlarge and rapidly progress to secretory lobules type 4. Even after post-lactational involution, the breast remains more differentiated. Mammary epithelial cells retain *in vitro* growth characteristics reflective of the degree of differentiation of the lobule from which they were derived. The identification of morphological *in vivo* and *in vitro* proliferative cell characteristics, response to hormones, and expression of gene products of differentiation are useful intermediate endpoints for assessing the potential of the breast for neoplastic transformation and its response to hormonal treatments leading to breast cancer prevention and therapy through induction of differentiation.

Key words: Breast cancer, carcinogenesis, hCG, pregnancy, prevention

Breast cancer in women has been recorded from ancient times. Egyptian papyri dating back to about 1600 BC make reference to this disease [1] which in modern industrialized societies has become the most frequent malignancy in women, with an incidence reaching epidemic proportions in the United States [2]. Although extensive multidisciplinary studies have provided a better un-

Address correspondence to José Russo, MD, Breast Cancer Research Laboratory, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

derstanding of its biology and combined therapeutic modalities have improved survival, breast cancer remains a lethal disease, second only to lung cancer as a cause of cancer-related death [2]. Failure to control this disease is attributed to its complexity. Central to it is the endocrinology of the female, which in its interaction with exogenous factors and lifestyle might contribute to increase or decrease in the incidence of breast cancer [2,3]. Important endocrinologic and reproductive factors associated with breast cancer include nulliparity or age older than 30 years at the first full-term pregnancy, early age at menarche, and late age at menopause. Reduced incidence, on the other hand, is associated with early parity, late menarche, and early menopause [3]. Additional factors such as diet and obesity also influence breast cancer risk; however, their role is still unclear, since they seem to affect premenopausal and postmenopausal women differently [4]. Familial history is increasingly important; genomic linkage searches are identifying women who have inherited dominant susceptibility genes, such as BRCA1, localized to chromosome 17q21, or BRCA2, to chromosome 13q-12–13 [5]. Sporadic breast cancers, however, represent almost 95% of all cases, and their genesis remains unknown.

Control of breast cancer requires a multidisciplinary approach. Surgical removal of the tumor has been the first line of breast cancer therapy since the first century AD [1]. Hormonal manipulation was not introduced until the end of the nineteenth century, when dependence of cancer on the ovary was proposed by Schinzinger in 1889 and demonstrated through the beneficial effects of oophorectomy by Beatson in 1896. This new era in cancer therapy did not begin until 1953, when Huggins advocated removal of the ovary as a source of estrogens [1]. Estrogens are believed to stimulate cell proliferation, which in turn leads to accumulation of random genetic errors that result in neoplasia. Based on this concept, one widely used approach to cancer control has been reducing the rate of cell division through administration of antiestrogenic compounds such as tamoxifen, progestagens, gonadotropin releasing hormone (GnRH) analogues and aromatase inhibitors [6]. Among these, tamoxifen has proven to be effective in both primary and advanced breast cancer.

In animal studies, tamoxifen interferes with the phases of tumor initiation and promotion [7]. Tamoxifen is species-, tissue-, and cell type-specific [8]. In the pubertal rat, tamoxifen promotes full ductal development in the mammary gland [9]; in the mature cycling animal it acts as an antiestrogen, causing atrophy of lobular structures [10]. Tamoxifen treatment in postmenopausal women results in upregulation of the proportion of ductal cells expressing estrogen receptor [11]. The use of tamoxifen in postmenopausal women has been advocated because of its beneficial effect in reducing blood lipids, ischemic heart disease, bone mineral metabolism and osteoporosis [12]. But it is also associated with the development of endometrial cancer [13], thromboembolic events [14], and ocular complications [15].

The unopposed-estrogen window hypothesis of breast cancer etiology has to a great extent guided these therapeutic approaches. However, breast epithelial cells are observed to exhibit maximal rates of proliferation during the progestational phase of the menstrual cycle; during the follicular phase, when estrogen levels prevail, proliferation is relatively low. Researchers have concluded that the combination of estrogen and progesterone appears to have a greater stimulatory effect than estrogen alone on cell division of the mammary epithelium [16]. This realization led other investigators to propose using GnRH analogues that inhibit ovulation and decrease the hormonal levels of premenopausal women to those of postmenopausal women [17].

Other chemopreventive agents proposed for use in breast cancer are monoterpenes [18] and retinoids [19]. Natural and synthetic vitamin A analogues, with their cyto-differentiating effect on acute promyelocytic leukemia cells [20], have inhibited mammary tumors induced by a variety of carcinogens [19]. This effect is manifested by decreased tumor incidence and multiplicity and increased latency of tumor development. However, the mechanism of action of retinoids on the mammary gland has not been clearly elucidated. It has been suggested that the chemopreventive efficacy of pharmacological doses of retinoids might relate to their observed inhibition of cell proliferation and the subsequent inhibition of morphologic development [19]. Based upon observations in animal models, a randomized clinical trial of retinoid efficacy on breast cancer chemoprevention is currently underway [21].

REPRODUCTION AND BREAST CANCER RISK

A full-term pregnancy completed before age 30 reduces the overall risk of breast cancer development by a magnitude of four-fold, and the protection is conferred for life [3,22]. Understanding the intimate mechanisms by which an early first full-term pregnancy in women confers protection against breast cancer is important, since it represents a physiological process which might induce architectural and cell kinetic changes such as those observed in experimental models [23–30] that make the organ refractory to cancer. This knowledge might have significant implications in our understanding of the biology of breast cancer, and more importantly, might serve as a physiological basis for developing strategies for breast cancer prevention and cure [31–34]. Our studies have focused on elucidating the pathogenesis of rat mammary cancer, identifying where malignant and benign lesions originate, and discovering factors that modulate the susceptibility of specific structures to neoplastic transformation [23–26]. Among these factors, pregnancy is a major modifier of the carcinogenic potential of the organ [27–30]; we have successfully used hormonal stimulation of virgin rats in an attempt to mimic the protective effect of pregnancy [31–34]. The knowledge gained from these studies served as the basis for testing the hypothesis that the human breast might exhibit similar developmental and cell kinetic characteristics which predispose it to neoplastic development [35].

DIFFERENTIATION AND MAMMARY CARCINOGENESIS

Our studies on the pathogenesis of rat mammary carcinomas induced by the chemical carcinogen 7,12-dimethylbenz(*a*)anthracene (DMBA) led us to conclude that the ability of the carcinogen to transform this organ is modulated by and inversely proportional to its degree of differentiation [29,30]. This postulate is supported by the observation that administering DMBA to 40–60 day-old young virgin rats induces the largest number of mammary tumors, whereas administering it to older or parous animals results in lower tumor incidence. The peak of maximal susceptibility, also called *susceptibility window*, coincides with the period in which the mammary gland contains numerous undifferentiated terminal ductal structures or terminal end buds (TEBs) actively cleaving into alveolar buds (ABs) [25,26]. The TEB is highly susceptible to neoplastic transformation due to the high proliferative rate of its epithelium, as determined by mitotic and DNA labeling indices [24–27]. These two indices are very high in the TEB and decrease toward the ductal or proximal portions of the gland, and in more differentiated structures such as ABs and lobules [24–27]. TEBs affected by the chemical carcinogen enlarge due to dilatation of the lumen and proliferation of the epithelial lining, which becomes multilayered or emits papillary projections; at this stage they are called intraductal proliferations (IDPs). IDPs progressively grow in size, and adjacent structures become confluent, leading to the formation of microtumors, histologically classified as intraductal carcinomas and invasive adenocarcinomas [23,30,35].

Mammary carcinogenesis is significantly inhibited when rats have completed one pregnancy with or without lactation prior to exposure to the carcinogen [24–26]. The protective effect observed after pregnancy appears to be permanent, since a delay of 21 or 63 days in the administration of DMBA does not ameliorate the inhibition of tumorigenesis [32]. This protection is attributed to permanent structural changes induced in the mammary parenchyma by reproductive processes [23,24,30,32]. The mechanism of the refractoriness of parous females, *vis-a-vis* young virgin and old virgin female rats, is due to decreased cell proliferation, decreased carcinogen binding, and increased DNA repair capability of the mammary epithelium, all of which result from the increased differentiation of the mammary gland [27,28].

Pregnancy represents a physiological, and therefore ideal, mechanism of mammary cancer prevention. Based on this knowledge, we hypothesized that induction of conditions that mimic pregnancy in virgin rats would result in similar reduction in cancer incidence. We have been able to mimic the protective effect exerted by pregnancy by treating young virgin rats with the placental hormone human chorionic gonadotropin (hCG) [31–33]. hCG Treatment of 50 day-old virgin rats for the length of pregnancy, 21 days, followed by administration of 8 mg DMBA/100 g body weight given by gastric gav-

age at 21 or 63 days after cessation of the hormonal treatment, reduced mammary carcinoma incidence in a dose-dependent manner [31–34]. Like pregnancy, hCG induced full differentiation of the mammary gland, morphologically manifested as a reduction in the number of TEBs due to a profuse lobular development, depression in DNA synthesis, inhibition of carcinogen binding to cellular DNA, and increased ability of the cells to repair DNA damage [32–34,36]. Once the inhibitory effect of hCG treatment on cancer initiation was proven, we tested the effect of the same hormonal treatment on tumor progression. Fifty day old virgin rats received the usual 8 mg DMBA/100 g body weight dose and were left undisturbed for 21 days in order to allow IDPs and early ductal carcinomas to develop. A daily treatment with 100 IU hCG was then initiated and maintained for 60 days. The hormonal treatment produced a significant reduction in the number of tumors and adenocarcinomas per animal in comparison with controls. These results indicate that hCG inhibits tumor progression by acting on cells already initiated in the neoplastic process [33,37]. The complex interactions of hCG with the ovarian follicle and *corpus luteum*, in which it stimulates the synthesis of estrogen, progesterone and inhibin, and with the mammary epithelium, in which it stimulates inhibin synthesis [36], are presently under intense investigation in our laboratory.

THE HUMAN BREAST

Studying the human breast requires defining the sex, age and reproductive history of the host before any description or definition of its architecture, development, function or hormone responsiveness are made. This is because the mammary gland lags behind other organs in its degree of development at birth, and no other organ presents such dramatic changes in size, shape and function as does the breast during growth, puberty, pregnancy and lactation [38]. Breast development study can be systematized by defining and quantitating various parenchymal components developed during different phases of body growth and sexual maturity [38]. Four different lobular structures, lobules type 1, 2, 3, and 4, have been characterized in the breast of postpubertal women, each representing one specific stage of breast development [38]. Type 1 lobules are most undifferentiated. Also called virginal lobules, they are present in the immature female breast before menarche; they are composed of clusters of 6 to 11 ductules per lobule. Lobules type 2 evolve from type 1 and have a more complex morphology with a higher number of ductular structures per lobule. They progress to lobules type 3, characterized by having an average of 80 ductules or alveoli per lobule; these are frequently seen in the breast tissue of women under hormonal stimulation or during pregnancy. The lobule type 4, which develops during the last trimester of pregnancy and remains present in the breast during the lactational period, is not found in prepubertal or nulliparous postpubertal women. The lobule type 4 is considered to be the maximal expression of parenchymal development and differentiation [38]. The breast tissue of nulliparous women is predominantly composed of more undifferentiated lobules type 1, although occasional lobules type 2 and 3 are present [39]. The architecture of the nulliparous woman's breast remains constant throughout her lifespan. The parous woman's breast has predominately lobule type 3, which peaks during the early reproductive years, decreasing after the fourth decade of life [39]. It is interesting to note that a history of parity between the ages of 14–20 years correlates with a significant increase in the number of lobules type 3, which remain as the predominant breast structure until age 40. After this age, the number of lobules type 3 decreases as they involute to predominantly lobules type 1 [39].

The importance of defining the various types of lobules present in the breast tissue of women of different ages and parity histories becomes evident when the presence of specific types are correlated with structures present in cancer bearing breasts [40]. Human breast cancer pathogenesis indicates that preneoplastic lesions, such as atypical ductal hyperplasias (ADH), which evolve to ductal carcinoma *in situ*, and progress to invasive carcinoma, originate in lobules type 1 [35]. Lobules type 2 are postulated to originate atypical lobular hyperplasia (ALH) and lobular carcinoma *in situ*, and lobules type 3 to be the site of origin of secretory adenomas, fibroadenomas, sclerosing adenosis and apocrine cysts. These observations indicate that the degree of differentiation or lobular development of the

mammary gland influences the type of tumors developed in the human breast [35].

An analysis of breasts which included the presence or absence of cancer in addition to parity revealed that breasts of nulliparous women without cancer and of nulliparous women with cancer had similar architectures. Both had lobule type 1 as the predominant structure, with a lower percentage of lobules type 2 and even lower type 3. In both groups the difference in relative percentage between lobules type 1 and 3 was highly significant [40]. The breast tissue of parous women without cancer contained predominantly lobules type 3 and a significantly lower percentage of lobules type 1 [40]. Parous women with breast cancer, on the other hand, had as the predominant structure the lobule type 1, with lower percentages of lobules type 2 and 3. These results indicated that the pattern of breast development in parous women who developed the disease was similar to that of nulliparous women. These results confirm our hypothesis that the degree of breast development is of importance in discerning susceptibility to carcinogenesis, and that parous women who develop breast cancer might exhibit a defective response to the differentiating effect of pregnancy hormones [36]. These findings indicate that the study of breast architecture through the quantitation and characterization of specific lobular types, indicators of the greatest level of differentiation achieved by the organ, provides valid endpoints for assessing breast differentiation. These parameters would, in turn, allow researchers to assess the risk of the gland to undergo neoplastic transformation when exposed to given genotoxic agents. In addition, they can be utilized as intermediate endpoints for assessing the effectiveness of chemopreventive or hormone preventive agents [36].

CONCLUSIONS

Our experimental system has allowed us to determine that pregnancy induces differentiation of the mammary gland, which results in protection of this organ from chemically induced carcinogenesis. The stimulus of pregnancy can be simulated in virgin animals by treatment with exogenous hormones, mainly the placental hormone hCG. Our studies found new evidence that hCG protects the mammary gland against carcinogenic initiation and progression, mimicking the physiological process of pregnancy without crippling other reproductive or endocrine functions. The importance of both differentiation and cell proliferation in tumor initiation and progression validates their use as endpoints for assessing the effect of hormones on the mammary gland prior to and after initiation of carcinogenesis. Collectively, our results provide solid bases for developing physiologic means of breast cancer prevention and control. The protection conferred by these processes, maintained even after the termination of either pregnancy or hormonal treatment, clearly indicates that differentiation induced by them is a permanent modification of the mammary glands' biological characteristics, even though the differentiated structures have regressed to seemingly more primitive conditions. Conclusions drawn in the experimental system have been validated for their application to the human situation through comparative studies in these two species.

ACKNOWLEDGEMENTS

Some of the breast samples utilized in these studies were generously provided by Dr. S.R. Bartow, obtained under contract NCI-RFP No. 1-CB-84231/NO1-CN-23928.

This research was supported by PHS grants CA48927, CA38921, CA06927, CA45538 awarded by the National Cancer Institute, NIH DHHS, and by American Cancer Society Grants CN52 and CN75291.

REFERENCES

1. Wagner FB Jr: History of breast disease and its treatment. In Bland KI, Copeland EM III (eds): "The Breast: Comprehensive Management of Benign and Malignant Diseases." Philadelphia: W.B. Saunders Co., 1991, pp 1–6.
2. Kelsey JL, Horn-Ross PL: Breast cancer: Magnitude of the problem and descriptive epidemiology. Epidemiol Rev 15:7–16, 1993.
3. Kelsey JL, Gammon MD, John EM: Reproductive factors and breast cancer. Epidemiol Rev 15:36–47, 1993.
4. Hunter DJ, Willett WC: Diet, body size and breast cancer. Epidemiol Rev 15:110–132, 1993.
5. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, Fields P, Marshall G, Narod S, Lenoir GM, Lynch H, Feunteun J, Devilee P, Cornelisse CJ, Menko FH,

Daly PA, Ormiston W, McManus R, Pye C, Lewis CM, Cannon-Albright LA, Peto J, Ponder BAJ, Skolnick MH, Easton DF, Goldgar DE, Stratton MR: Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 265:2088–2090, 1994.

6. Henderson BE, Ross RK, Pike MC: Hormonal chemoprevention of cancer in women. Science 259:633–638, 1993.
7. Jordan VC, Allen KE: Evaluation of the anti-tumor activity of the nonsteroidal antiestrogen monohydroxytamoxifen in the DMBA-induced rat mammary carcinoma model. Eur J Cancer Clin Oncol 16:239–251, 1980.
8. Furr BJ, Jordan VC: The pharmacology and clinical uses of tamoxifen. Pharmacol Ther 25:127–205, 1984.
9. Nicholson RI, Gotting KE, Gee J, Walker KJ: Actions of oestrogens and anti-oestrogens on rat mammary gland development: Relevance to breast cancer prevention. J Steroid Biochem 30:95–103, 1988.
10. Gotze S, Nishino Y, Neumann F: Anti-oestrogenic effects of tomoxifen on mammary gland and hypophysis in female rats. Acta Endocrinol 105:360–370, 1984.
11. Walker KJ, Price-Thomas JM, Candlish W, Nicholson RI: Influence of the antiestrogen on normal breast tissue. Br J Cancer 64:764–768, 1991.
12. Wright CDP, Mansel RE, Gazet J-C, Compston JE: Effect of long term tamoxifen treatment on bone turnover in women with breast cancer. Br Med J 306:429–430, 1993.
13. Fornander T, Rutqvist LE, Cedemark B, Glas U, Mattsson A, Silfverswärd C, Skoog L, Somell A, Theve T, Wilking N, Askergren J, Hjalmar M-L: Adjuvant tamoxifen in early breast cancer: Occurrence of new primary cancers. Lancet i:117–120, 1989.
14. Jones AL, Powles TJ, Treleaven JG, Burman JF, Nicolson MC, Chung H-I, Ashley SE: Hemostatic changes and thromboembolic risk during tamoxifen therapy in normal women. Br J Cancer 66:744–747, 1992.
15. Pavlidis N, Petris C, Briassoulis E, Klouvas G, Psilas C, Rempapis J, Petroutsos G: Clear evidence that long-term, low-dose tamoxifen treatment can induce ocular toxicity. Cancer 69:2961–2964, 1992.
16. Potten CS, Watson RJ, Williams CT, Tickle S, Roberts SA, Harris H, Howell A: The effect of age and menstrual cycle upon proliferative activity of the normal human breast. Br J Cancer 58:163–168, 1988.
17. Spicer D, Shoupe D, Pike MC: Gonadotropin-releasing hormone agonist plus add-back sex steroids to reduce risk of breast cancer. J Natl Cancer Inst 83: 121–213, 1991.
18. Gould MN: Chemoprevention of mammary cancer by monoterpenes. Proc Am Assoc Cancer Res 34:572–573, 1993.
19. Welsch CW, Zile MH, Cullum ME: Retinoids and mammary gland tumorigenesis: A critique. In Reddy BS, Cohen LA (eds): "Diet, Nutrition and Cancer: A Critical Evaluation." Boca Raton: CRC Press, 1986, pp 1–21.
20. Miller, WH Jr, Dmitrovsky E: Retinoic acid and its rearranged receptor in the treatment of acute premyelocytic leukemia. In DeVita V, Hellman S, Rosenberg SA (eds): "Important Advances in Oncology." Philadelphia: J.B. Lippincott Company, 1993, pp 81–90.
21. Costa A: Breast cancer chemoprevention. Eur J Cancer 29A:589–592, 1993 (abstract).
22. MacMahon B, Cole P, Liu M, Lowe CR, Mirra AP, Ravinihar B, Salber EJ, Valaoras VG, Yuasa S: Age at first birth and breast cancer risk. Bull World Health Organ 43:209–221, 1970.
23. Russo J, Saby J, Isenberg W, Russo IH: Pathogenesis of mammary carcinomas induced in rats by 7,12-dimethylbenz(*a*)anthracene. J Natl Cancer Inst 59:435–445, 1977.
24. Russo IH, Russo J: Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenz(*a*)anthracene. J Natl Cancer Inst 61:1439–1449, 1978.
25. Russo J, Russo IH: DNA labeling index and structure of the rat mammary gland as determinants of its susceptibility to carcinogenesis. J Natl Cancer Inst 61: 1451–1459, 1978.
26. Russo J, Wilgus G, Russo IH: Susceptibility of the mammary gland to carcinogenesis. I. Differentiation of the mammary gland as determinant of tumor incidence and type of lesion. Am J Pathol 96:721–734, 1979.
27. Russo J, Russo IH: Influence of differentiation and cell kinetics on the susceptibility of the mammary gland to carcinogenesis. Cancer Res 40:2677–2687, 1980.
28. Russo J, Tay LK, Russo IH: Differentiation of the mammary gland and susceptibility to carcinogenesis: A review. Breast Cancer Res Treat 2:5–73, 1982.
29. Russo J, Russo IH: Is differentiation the answer in breast cancer prevention? IRCS J Med Sci 10:935–941, 1982.
30. Russo J, Russo IH: Biological and molecular bases of mammary carcinogenesis. Lab Invest 57:112–137, 1987.
31. Russo IH, Koszalka MS, Russo J: Protective effect of chorionic gonadotropin on DMBA-induced mammary carcinogenesis. Br J Cancer, 62:243–247, 1990.
32. Russo IH, Koszalka M, Russo J: Comparative study of the influence of pregnancy and hormonal treatment on mammary carcinogenesis. Br J Cancer 64: 481–484, 1991.
33. Russo IH, Koszalka M, Russo J: Human chorionic gonadotropin and rat mammary cancer prevention. J Natl Cancer Inst 82:1286–1289, 1990.
34. Russo IH, Koszalka M, Russo J: Effect of human chorionic gonadotropin on mammary gland differentiation and carcinogenesis. Carcinogenesis 11:1849–1855, 1990.
35. Russo J, Gusterson BA, Rogers AE, Russo IH, Wellings SR, van Zwieten MJ: Comparative study of human and rat mammary tumorigenesis. Lab Invest 62:244–278, 1990.

36. Russo IH, Russo J: Role of hCG and inhibin in breast cancer (review). Int J Oncol 4:297–306, 1994.
37. Russo J, Russo IH: Toward a physiological approach to breast cancer prevention. Cancer Epidemiol Biomarkers Prev 3:353–364, 1994.
38. Russo J, Russo IH: Development of the human mammary gland. In Neville MD, Daniel C (eds): "The Mammary Gland." New York: Plenum Publishing Inc 1987 pp 67–93.
39. Russo J, Rivera R, Russo IH: Influence of age and parity on the development of the human breast. Breast Cancer Res Treat 23:211–218, 1992.
40. Russo J, Romero AL, Russo IH: Architectural pattern of the normal and cancerous breast under the influence of parity. Cancer Epidemiol Biomarkers Prev 3:219–224, 1994.