Mammary Gland Architecture as a Determining Factor in the Susceptibility of the Human Breast to Cancer

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Abstract: The developmental pattern of the breast can be assessed by determining the composition of the breast in specific lobular structures, which are designated as lobules type 1 (Lob 1), lobules type 2 (Lob 2), and lobules type 3 (Lob 3), with Lob 1 being the less developed and Lob 3 being the most differentiated or with the highest number of ductules per lobular unit. In the present work, the patient population consisted of three groups of women who underwent surgical procedures: The first group included women who underwent reduction mammoplasty (RM) for cosmetic reasons. The second group included women who underwent prophylactic subcutaneous mastectomy after genetic counseling for either carrying the BRCA-1 gene or belonging to a pedigree with familial breast cancer (FAM), and the third group included women who underwent modified radical mastectomy (MRM) for the diagnosis of invasive carcinoma. The RM group consisted of 33 women, of whom 9 were nulliparous and 24 were parous. The FAM group consisted of 17 women, of whom 8 were nulliparous and 9 were parous. The MRM group consisted of 43 women, of whom 7 were nulliparous and 36 were parous. The analysis of the lobular composition of all of the samples from the RM group, which is considered the control group, revealed that Lob 1 represented 22%, Lob 2 represented 37%, and Lob 3 represented 38%, whereas the tissue examined from the FAM and MRM groups contained a preponderance of Lob 1 at 48% and 74%, respectively, over Lob 3, which was 10% and 3%, respectively. When the results of the analysis of breast tissue were separated according to the pregnancy history of the donor, it was found that in the control group or RM, there was a significant difference in lobular composition. Nulliparous women of the RM group showed a preponderance of Lob 1 (46%) over parous women, which contained only 17%, whereas the percentage of Lob 3 in the nulliparous group was significantly lower (7%) than the parous group (48%). In the breast tissues obtained from FAM and MRM, no significant differences in lobular composition were observed, as all of the samples contained a higher concentration of Lob 1, independent of the pregnancy history. The breast tissue of FAM and MRM of parous women had a developmental pattern that was similar to that of nulliparous women of the same group and that was less developed than the breast of parous women of the control group. An important difference between the Lob 1 of the FAM group versus the control (RM) and the MRM group was that most of these lobules had thin ductules with an increase in hyalinization of the intralobular stroma manifested in the whole-mount preparation as an alteration in the branching pattern. The data suggest that the breast tissue of women with invasive cancer, as well as those from a background of familial breast cancer, have an architectural pattern different from the control or normal tissues and that the BRCA-1 or related genes may have a functional role in the branching pattern of the breast during lobular development, mainly in the epithelial stroma interaction.

Key Words: breast cancer, BRCA-1, lobular development, stroma

The incidence of breast cancer has gradually increased in the United States and in most Western
societies over the last few decades (1). Although the reasons for this increase are not certain, epidemiological, clinical, and experimental data indicate that the risk of developing breast cancer is strongly dependent on endocrine conditions modulated by ovarian function, such as early menarche, late menopause, and parity (1–5). Age is another important risk factor because breast cancer, which is practically nonexistent before age 24, exhibits maximal incidence during the postmenopausal years (1–5). The majority of breast cancer patients are women in the sixth and seventh decades of life, and the mortality for breast cancer also continues to rise after menopause. The age-specific incidence, that is, the number of cases per year per 100,000 women in each age group, climbs rapidly after the age of 30 years, reaching a peak of maximal incidence of 500 cases per 100,000 women in the 60- to 70-year-old group (1). The fact that women who had given birth to a child when they were younger than 24 years of age exhibited a decrease in their lifetime risk of developing breast cancer and that additional pregnancies increase the protection (6) adds complexity to this paradox. A plausible explanation for the lifetime protective effect of an event occurring so early in life is provided by the biological behavior of breast cancer and by comparative studies with experimental animal models. Epidemiological observations indicate that a higher breast cancer incidence occurs in women who had been irradiated, but only in those in whom exposure occurred at a young age, namely before 19 years of age, but not in those that were irradiated at older ages or after pregnancy (7). In rodents, the maximal incidence of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary cancer occurs when the carcinogen is administered to young virgin cycling rats, but the same carcinogen fails to induce tumors when given to rats after a full-term pregnancy (8–10). The high susceptibility of the young virgin rat mammary gland in developing malignancies is the result of the interaction of the carcinogen with rapidly dividing cells present in terminal end buds (TEBs), undifferentiated structures that represent the most active growth centers of the mammary parenchyma. Cancer initiation in this model is the result of a combination of a high rate of carcinogen binding to the epithelial DNA, fixation of transformation, formation of polar metabolites, and deficient DNA repair (11–16), among other factors. Although no specific etiologic agent of breast cancer has been identified, there are close similarities between the pathogenesis of this disease in women and that induced in rodents by chemical carcinogens. Ductal carcinoma, the most common breast malignancy, originates in lobules type 1 (Lob 1), also called the terminal ductal lobular unit, an undifferentiated structure that is considered to be equivalent to the TEB, the site of origin of ductal carcinomas in rodents (17–21). The validity of this comparison is further strengthened by the demonstration that under in vitro conditions, human breast epithelial cells can be transformed by the same chemical carcinogens that induce mammary cancer in experimental animals (19,22). These observations suggest that if the human breast is exposed to a carcinogenic insult, the Lob 1 would be the structure affected and thus be responsible for the initiation of a malignancy (17). Therefore, it is possible to postulate that genomic damage caused by radiation, environmental carcinogens, hormonal imbalances, and/or other still unidentified factors, either alone or in combination with genetic predisposition, might be responsible of the causation of breast cancer in women. For cancer to develop, however, this multifactorial combination must occur during the window of high susceptibility that is encompassed between menarche and the first full-term pregnancy (FFTP), even though the cells that became damaged would be clinically detectable as a neoplasm at premenopause or postmenopause, after several years of progressing along the various stages of transformation (19,22,23). An understanding of how these multifactorial influences lead to genomic damage and cancer initiation requires identifying within the breast the foci of high susceptibility to be transformed. This objective is marred by innumerable difficulties because the understanding of human breast development has been and continues being a major biological puzzle. The fact that the mammary gland is one of the few organs that is not fully developed at birth represents the first difficulty (24,25). No other organ presents such dramatic changes in size, shape, and function as does the breast during growth, puberty, pregnancy, lactation, and postmenopausal regression (24,26–29). The development of the breast starts as early as the stage of nipple epithelium during embryonic life, continuing after birth in parallel with body growth. A spurt of growth with lobule formation occurs at puberty, but the completion of breast development and differentiation occurs only at the end of a full-term pregnancy (24,29). Thus, as long as a woman does not become pregnant, the predominant structure in her breast would be the Lob 1. With pregnancy, the mammary parenchyma reaches the final stage of secretory lobule type 4 (Lob 4), which forms by the end of the reproductive process and remains present during lactation. Despite regression after weaning, the breast of parous women retains until
menopause the more differentiated lobule type 3 (Lob 3) as the predominant structure. Lob 3 has a lower rate of cell proliferation and steroid hormone receptor content than Lob 1. Lob 3 also expresses several genes that are related to the differentiation process (30-32), such as a new serpin gene (31), mammary-derived growth factor inhibitor (32), and genes controlling programmed cell death and DNA repair. The protection conferred by pregnancy has been in great part explained by our studies of DMBA-induced carcinogenesis. In this model, an almost complete abolition of the oncogenic response to DMBA results from the induction of differentiation of the mammary gland by either full-term pregnancy or treatment of virgin rats with human chorionic gonadotropin (hCG), one of the main hormones produced during pregnancy (9,23). Our studies led us to conclude that both parity and the hormones of pregnancy in the absence of conception are important modulators of the pattern of mammary lobular development and differentiation, cell proliferation, steroid hormone receptor content, and gene expression.

The painstaking process of identifying the mRNA, cDNA, and protein expression of specific genes has been greatly simplified and accelerated by the comparative analysis of gene expression utilizing a microarray system. We have compared gene expression in breast epithelial cells obtained from Lob 1 and Lob 3 of nulliparous and parous women, respectively. Their analysis, utilizing a nylon filter membrane that contains 1176 genes, revealed that the more differentiated Lob 3 genes that have never been considered relevant in the normal breast are upregulated, thus providing a new insight in their role in the differentiation pattern of this organ. It is of interest to note that known genes that are overexpressed in breast cancer, such as HER-2/neu (33) and mucin (34,35), were not expressed in any of the lobular structures. In addition, cells derived from the differentiated Lob 3 are resistant to growth in vitro and do not express transformation phenotypes upon carcinogen treatment, as do cells from Lob 1 (19,22,24,25).

We postulate that the induction of differentiation of the breast by the reproductive event is responsible for the inhibition of carcinogenic initiation, as it has been observed in the rodent experimental model (9,10,13,14, 20,23,36) and in humans (1,3,19,37). However, a certain percentage of parous women develop breast cancer (6). We have observed that the architectural pattern of breast tissues from parous breast cancer-affected patients appears similar to that of nulliparous women, having Lob 1 as the prevalent structure. These characteristics indicate that the breast in these women has responded to the hormones of pregnancy differently than the breast of parous women who did not develop cancer (20,38,39). The possibility that these breast tissues exhibit a defective response to the differentiating influence of the hormones of pregnancy warrants further investigation. The pattern of breast development and differentiation is also influenced by inheritance (20). Genetic influences, which are responsible for at least 5% of the breast cancer cases, also seem to affect the architectural pattern of the breast.

The studies of prophylactic mastectomy specimens obtained from women with a familial history of breast and breast/ovarian cancer or from women proven to be carriers of the BRCA-1 gene by linkage analysis have revealed that morphologically and architecturally the breasts of nulliparous women are similar to those of parous women. Breast tissues from both groups of women are predominantly composed of Lob 1, with only a few specimens containing Lob 2 and Lob 3, in frank contrast to the predominance of Lob 3 found in parous women without a familial history of breast cancer (20,25). These observations suggest that genetic predisposition to breast cancer affects genes that control the branching pattern of the breast during lobular development. Therefore, although there is no explanation as yet for the higher breast cancer risk exhibited by nulliparous, late parous, and genetically predisposed women, the fact that experimentally induced rat mammary carcinomas develop only when the carcinogen interacts with the undifferentiated and highly proliferating mammary epithelium of young nulliparous rats (10,13,15,36) suggests that the breast of these “high breast cancer risk” women might exhibit some of the undifferentiated cell characteristics that have been shown to be essential for the initiation of cancer in rodents. The higher proliferative activity of the nulliparous woman with Lob 1, in association with the higher breast cancer incidence in this group of women, suggests that these lobules are biologically different from those of early parous women (19,21,22,24,25,30).

In the present work, we present data supporting the concept that the developmental mechanisms through which pregnancy and hormonally induced differentiation affect the mammary gland, determine the susceptibility to carcinogenesis.

ARCHITECTURE OF THE NORMAL HUMAN BREAST

The human breast is one of the few organs of the body that is not completely developed at birth. It
progressively develops from infancy to puberty under the main stimuli of pituitary and ovarian hormones. A fully differentiated condition is reached by the end of a full-term pregnancy, under the stimulus of new endocrine organs, the placenta, and the developing fetus. These new hormonal influences induce a profuse branching of the mammary parenchyma leading to the formation of secretory lobular structures (21,24,25).

The least differentiated structure identified in the breast of postpubertal nulliparous women is the Lob 1, which is composed of clusters of 6–11 ductules per lobule. Lob 1 progresses to Lob 2, which is composed of more numerous ductules per lobule and exhibits a more complex morphology. In the first and second trimesters of pregnancy, Lob 1 and Lob 2 rapidly progress to Lob 3, which is composed of an average of 80 ductules or small alveoli per lobule. During the last trimester of pregnancy, they reach the final stage of secretory Lob 4. When active milk secretion supervenes, the alveoli become distended, a characteristic of the Lob 4 that is present during the lactational period. After weaning, all of the secretory units of the breast regress, reverting to Lob 3 and Lob 2 (22).

**CELL PROLIFERATION AND STEROID RECEPTOR CONTENT IN THE NORMAL BREAST**

The initial classification of lobules into four categories was primarily based on morphological characteristics of these structures (21,24,25). Further analysis led us to discover variations in the rate of cell proliferation among these categories. Cell proliferation, a cellular function essential for normal growth, also plays a crucial role in the development of cancer (30,40–42). Normal growth requires a net increase of cycling cells over two other cell populations, resting cells (arrested in G0) and dying cells (cells lost through programmed cell death or apoptosis). The quantitation of proliferating cells, determined by immunocytochemical reaction of breast tissues of nulliparous and parous women with monoclonal antibody against Ki67 antigen, allowed us to determine that the proliferative activity of the mammary epithelium varies as a function of the degree of lobular differentiation. The percentage of Ki67-positive cells (Ki67 or proliferation index) decreases progressively as the lobules mature from Lob 1 to Lob 2 and these to Lob 3 and Lob 4 (30,40–42). These differences were not abrogated when the proliferation index was corrected for the phase of the menstrual cycle (41,42). We concluded that parity, in addition of exerting an important influence in the lobular composition of the breast, profoundly influences the proliferative activity of the breast.

Estrogens and progesterone are known to promote proliferation and differentiation in the normal breast. Both steroids act intracellularly through their respective nuclear receptors, which become activated by the binding of the hormone. This mechanism regulates the expression of specific genes and is one of the accepted modes of action of estrogens for inducing cell proliferation (43,44). However, the mechanisms by which these molecules exert their mitogenic and differentiation effects have not been clearly established (45–53). We have measured the levels of steroid hormone receptors in normal breast tissue by identifying and quantitating individual cells expressing the traditional estrogen receptor (ER), now called ERα, and progesterone receptor (PgR) using monoclonal antibodies that specifically recognize these receptors (30,40–42). Our studies revealed that the percentage of cells expressing these receptors varies as a function of the degree of lobular development of the breast and therefore of the type of lobular structure analyzed. Lob 1 consistently contains a higher percentage of ERα and PgR-positive cells than Lob 2, Lob 3, and Lob 4, indicating that a progressive decrease in the percentage of cells exhibiting an immunocytochemically positive reaction for these markers occurs as the structures become more differentiated (42). The facts that proliferative activity and percentage of cells positive for both ERα and PgR are highest in Lob 1, and they progressively decrease in an inverse relationship to the degree of lobular differentiation and provide a mechanistic explanation for the higher susceptibility of these structures to be transformed by chemical carcinogens in vitro (19,22).

**INFLUENCE OF AGE AND PARITY ON HUMAN BREAST DEVELOPMENT**

The development of breast cancer is heavily influenced by the reproductive history of the individual, as women with a history of early pregnancy are at lower risk of developing breast malignancies (3,4,54). Because our studies of chemically induced carcinogenesis in an experimental animal model have shown that the initiation of the neoplastic process is inversely related to the degree of differentiation of the mammary gland, which in turn is a function of age and reproductive history (6,9,17,55), we tested the hypothesis that the protective effect of early FFTP in women retains the more differentiated lobules of the breast (19,22). For
this purpose, we analyzed breast samples obtained from bilateral or unilateral reduction mammoplasties in order to determine the quantity and type of parenchymal structures present in them.

The comparison between the architecture of the breast of nulliparous and parous women revealed that the breasts of nulliparous women contained almost exclusively Lob 1, in whom the percentage remained nearly constant throughout the life span of the individual, whereas the same structure in the parous women was in a very low percentage during the younger years, starting to increase after the fourth decade of life and more significantly after menopause, when they reached the same level observed in nulliparous women. This latter group of women never reached the degree of differentiation found in women who completed an early pregnancy. Even though during the postmenopausal years in the breast of both parous and nulliparous women the preponderant structure is the Lob 1, only the nulliparous women are at high risk of developing breast cancer, whereas parous women remain protected (24). Because ductal breast cancer originates in Lob 1 (17,18), the epidemiological observation that nulliparous women exhibit a higher incidence of breast cancer than parous women (3,4) indicates that Lob 1 in these two groups of women might be biologically different or exhibit different susceptibility to carcinogenesis (19,24,25). The presence of Lob 1 in the breasts of parous women has also been interpreted as a failure of the mammary parenchyma to respond to the influences of pregnancy and lactation (25). Therefore, it is possible to postulate that unresponsive lobules that fail to undergo full differentiation under the stimuli of pregnancy and lactation are responsible for cancer development despite the parity history of a woman. If this is the case, then these unresponsive Lob 1 would be as sensitive to carcinogenesis as the lobules found in the breasts of nulliparous women. Further studies are required in order to determine whether gene expression in those lobules is different. This “molecular phenotype” will shed light on the functional differences between cells and tissues that may be not obvious at morphological level. Furthermore, we expect that a broad mapping of gene expression of early parous women free of cancer in comparison with that of nulliparous and late parous women with cancer will allow us to identify the differences postulated here. Genomic mapping will significantly add to our observations of lower proliferative activity in the Lob 1 of the parous woman’s breast, whereas cell proliferation is higher in the Lob 1 of the nulliparous woman’s breast (40–42). We have also shown that during the fourth and fifth decades of

**Figure 1.** Histological section of a lobule type 1, hematoxylin and eosin. ×25. (a) Lob 1 with a good demarcation between the intralobular and interlobular stroma. (b and c) Lob 1 with increase density in the intralobular stroma. (d) Lob 1 with marked fibrosis in the intralobular stroma and lack of demarcation from the extralobular stroma.
life, there is a decrease in the number of Lob 2. We postulate that this type of lobule is the site of origin of both lobular hyperplasia and carcinoma in situ (17,21). Because it has been reported that the incidence of atypical lobular hyperplasia decreases significantly with advancing age (56), it is possible to postulate that the observed diminution in Lob 2 is responsible for the decreased incidence of this type of lesions. In addition to the differences in proliferative activity, the three types of lobules exhibit variations in their in vitro growth characteristics. Lob 1 and Lob 2 grow faster, have a higher DNA labeling index and a shorter doubling time than Lob 3 (57). They also exhibit different susceptibility to carcinogenesis (19,22). Cells obtained from Lob 1 and Lob 2 express in vitro phenotypes that are indicative of neoplastic transformation when treated with chemical carcinogens, whereas cells obtained from Lob 3 did not manifest those changes (19,22). In the experimental system, we have found that pregnancy as well as differentiation of the gland induced by the placental hormone hCG follows a pattern of differentiation similar to the one reported in this work (23,57,58). The data discussed previously here are further supported by this experimental system, in which it has been observed that full-term pregnancy as well as treatment of virgin rats with hCG induces differentiation of the mammary gland, which results in protection from chemically induced carcinogenesis. The stimulus of pregnancy or of exogenous hormones further the differentiation of the TEB, stimulating lobular development (57,58).

PARENCHYMA-STROMA RELATIONSHIP IN THE NULLIPAROUS AND PAROUS BREAST

Breast development occurs through a process of ductal elongation, branching, and sprouting of ductules or alveoli, a process that requires extensive cell proliferation and penetration of the ductal epithelium into the stroma (24). Both the intralobular and the interlobular stromata are affected simultaneously during development, pregnancy, lactation, and involution (24,59,60). These processes occur, in turn, in a synchronous manner in response to specific hormonal and growth factor stimuli (29). Two major mechanisms are considered to be involved in the interaction of the stroma and epithelial cells: the production of soluble growth factors and a modification of the composition of the extracellular matrix. This interaction seems to be bidirectional such that epithelial cells are also capable of influencing stromal cell behavior and governing gene expression (59,60). The study of the stroma-parenchyma ratio in 14 breasts of pubertal, postpubertal, parous, and pregnant women showed that the relationship between parenchyma and stroma is a dynamic process. At puberty, almost 90% of the mammary gland is made up of stroma, the intralobular stroma, which represents 17% of the total, and consists of the connective tissue that surrounds each individual alveolar bud, and the interlobular stroma, composed of fat and connective tissue, which separates one lobule from another. The parenchyma of these glands, representing 10% of the mammary area, is made up almost exclusively of Lob 1 and ductal structures. In the glands of postpubertal and young nulliparous women, the parenchyma increases from 10 to 30% of the total area of the gland (0–10% is composed of Lob 1, 10–18% of Lob 2, and 1–3% of Lob 3). The intralobular stroma of these breasts represents approximately 28% of the total (25). Parity induces significant differences in mammary gland development. The breast of parous women is mostly composed of Lob 3 and accounts for 24% of the total parenchyma and a markedly reduced proportion of Lob 1 and Lob 2. Pregnancy induces dramatic changes in the parenchyma-stroma ratio. During the process of postlactational involution, Lob 4 regresses to Lob 3. These lobules represent almost 60% of the structures of the parous breast until the fourth decade of life, when Lob 2 and Lob 1 become preponderant structures and the stroma regains its prevalence. It is expected that the marked variations in epithelial-stromal ratio occurring during the various stages of breast development will influence the bidirectional connections between the components of cellular microenvironment (growth factors, hormones, and extracellular matrix) and the nucleus, leading to specific modifications in gene expression that may account for the different susceptibility or risk to develop breast cancer.

BREAST DEVELOPMENT AND THE PATHOGENESIS OF BREAST CANCER

Lob 1, the most undifferentiated structure found in the breast of young nulliparous women, is the site of origin of ductal carcinomas (10,36). The finding that the most undifferentiated structures originated the most aggressive neoplasms lends support to our hypothesis that the presence of Lob 1 explains the higher breast cancer risk of nulliparous women, as they represent the population with the highest concentration of undifferentiated structures in the breast (21). In order
to further the demonstration of this postulate, we have compared the breast tissue of three groups of women who underwent surgical procedures: The first group consisted of women who underwent reduction mammoplasty (RM) for cosmetic reasons. The second group included women who underwent prophylactic subcutaneous mastectomy after genetic counseling for either carrying the BRCA-1 gene or belonging to a pedigree with familial breast cancer (FAM), and the final group included women who underwent modified radical mastectomy (MRM) for the diagnosis of invasive carcinoma (Table 1).

The breasts of women who underwent RM contain the three types of lobules that follow the same morphologic characteristics that are described previously (61). The three lobular structures are in general surrounded by a loose stroma that demarcates them from the interlobular stroma that may have a different ratio of connective and fat tissue (Fig. 1a). All of the lobules were very well demarcated, and no fibrous tissue was observed (Fig. 1a and Table 2). Quantification of the three lobular structures in the overall population of breast tissue studied indicated that lobules type 1 represented 22.5% of the structures, whereas Lob 2 was 37.3% and Lob 3 was 38.4% of the total number of structures. The differences are statistically significant (Table 1). The separation of the breast samples, based on the pregnancy history of the host, such as nulliparity and parity, showed a different pattern of lobular development. The breast of nulliparous women contained a significantly higher number of Lob 1 and Lob 2, with 45.9% and 47.2%, respectively, and a highly significantly lower number of Lob 3 (6.9%; Table 1). In the breast of parous women, the pattern was inverse, with Lob 2 and Lob 3 being the most abundant at 35.5% and 47.9%, respectively, whereas Lob 1 comprised only 16.9% of the total.

The breasts of women with familial breast cancer were obtained from prophylactic mastectomies. Seventeen samples that have been verified to be either BRCA-1 or a carrier of genetic abnormalities are the ones entered in this analysis (Table 3). The average age of these women was 37.0 ± 2.9 (Table 1). The histological appearance of the lobular structures was different from that observed in the breast tissue of women who underwent RM. Eight out 17 breast samples presented a well-demarcated lobular structures, but all of them had moderate or marked fibrosis of the intralobular stroma (Fig. 1b–d and Table 2). Ductal hyperplasia (mild to severe) in the Lob 1 or Lob 2 was observed in seven cases and carcinoma in situ (solid, cribriform, and papillary) in one case, and invasive carcinomas ipsilaterally or contralaterally was observed in nine cases (Table 4).

The distribution of Lob 1, Lob 2, and Lob 3 in the breast tissue derived from women with BRCA-1, or being carriers of genetic abnormalities by linked analysis was 47.9%, 39.9%, and 9.9%, respectively (Table 1).

| Table 1. Lobular Architecture of the Breast Tissue from RM, Prophylactic Mastectomy for FAM, and MRM |
| Sets | Number of cases | Age Mean ± SD (%) | Lob 1 Mean ± SD (%) | Lob 2 Mean ± SD (%) | Lob 3 Mean ± SD (%) |
|------|-----------------|--------------------|---------------------|---------------------|---------------------|
| RM (All) | 33 | 29.4 ± 8.2 | 22.5 ± 23.7 | 37.3 ± 28.6 | 38.4 ± 34.2 |
| RM (Nulliparous) | 9 | 22.9 ± 6.7 | 45.9 ± 27.4 | 47.2 ± 22.0 | 6.9 ± 7.0 |
| RM (Parous) | 24 | 31.9 ± 2.3 | 16.9 ± 8.3 | 35.5 ± 3.1 | 47.9 ± 33.4 |
| FAM (All) | 17 | 37.0 ± 2.9 | 47.9 ± 37.3 | 39.9 ± 31.3 | 9.91 ± 4.41 |
| FAM (Nulliparous) | 17 | 37.6 ± 3.2 | 51.3 ± 34.4 | 39.9 ± 32.6 | 8.83 ± 3.9 |
| FAM (Parous) | 9 | 36.5 ± 2.6 | 44.0 ± 42.0 | 40.0 ± 38.1 | 16.10 ± 9.9 |
| MRM (All) | 43 | 35.4 ± 3.9 | 74.3 ± 25.8 | 22.3 ± 22.1 | 3.35 ± 10.0 |
| MRM (Nulliparous) | 7 | 36.0 ± 3.6 | 80.0 ± 19.0 | 16.8 ± 15.0 | 1.74 ± 4.6 |
| MRM (Parous) | 36 | 35.2 ± 4.3 | 70.4 ± 26.4 | 25.4 ± 22.7 | 3.80 ± 12.5 |

The lobular structures of both parous and nulliparous women, considered as a group, showed that Lob 1 of women with reduction mammoplasty (RM) was significantly lower than those of the FAM and MRM group (t = 7.6, p < 0.0003, and t = 11.0, p < 0.00000, respectively). The percentage of Lob 1 between the FAM and MRM was also significantly different (t = 3.38, p < 0.001). Lob 2 was significantly different between the RM and FAM (t = 3.58, p < 0.005) and between FAM and MRM (t = 2.20, p < 0.02). Lob 3 was higher in the RM group than the FAM and MRM group (t = 3.83, p < 0.002, and t = 8, p < 0.0000, respectively). Lob 3 was also different between FAM and MRM (t = 2.17, p < 0.02). In the RM group, nulliparous Lob 1 was significantly different from Lob 3 (t = 3.43, p < 0.008) and between Lob 2 and Lob 3 (t = 5.46, p < 0.0005). In the parous group, Lob 1 was significantly different from Lob 2 and Lob 3 (t = 2.78, p < 0.009, and t = 3.82, p < 0.0000, respectively). In the FAM group, nulliparous Lob 1 was significantly different from Lob 3 (t = 2.52, p < 0.03) and between Lob 2 and Lob 3 (t = 3.41, p < 0.007). In the parous group, Lob 1 was significantly different than Lob 2 and Lob 3 (t = 3.00, p < 0.005, and t = 3.86, p < 0.0002). Lob 2 was significantly different from Lob 1 (t = 2.7, p < 0.007). In the parous group, Lob 1 was significantly different from Lob 2 and Lob 3 (p < 0.005 and p < 0.001, respectively, and between Lob 2 and Lob 3, p < 0.001). For groups RM and FAM, an average of 100 g of tissue were processed from every specimen. The tissues were dehydrated, cut into 1 mm thick slices, which were mounted on glass slides and coveredipped. Histological sections were prepared from paraffin embedded tissue sectioned at 5 μm each and stained with hematoxylin and eosin. An average of 12 slides per sample was examined, and 2,826 structures were classified and counted. All of the whole mounts and histological sections were examined under a stereomicroscope (Olympus SZH) or a bright field Olympus microscope (BHT-2), respectively, for classification and quantification. For the MRM group, all tissues were fixed in formalin, embedded in paraffin, sectioned at 5 μm thickness, and processed for light microscopy. An average of 12 histological sections per sample was examined. These histological sections were utilized for characterizing the type of lobular structures and the number and type of pathological lesions present by applying criteria previously described (24).
Table 2. Profile of the Lobular Structures in the Breast Tissues Obtained from RM, Prophylactic Mastectomy for FAM, and MRM for Invasive Cancer

| Group | Number of cases | Number of well-defined lobules (%) | Number of poorly defined lobules (%) | Number of none (%) | Fibrosis mild to moderate (%) | Fibrosis marked (%) |
|-------|----------------|----------------------------------|-------------------------------------|-------------------|-------------------------------|------------------|
| RM    | 33             | 33 (100)                         | 0 (0)                               | 33 (100)          | 0                             | 0                |
| FAM   | 17             | 8 (47)                           | 9 (53)                              | 0                 | 3 (17.6)                      | 14 (82.4)        |
| MRM   | 43             | 40 (93)                          | 3 (7)                               | 1 (2.3)           | 39 (90.3)                     | 3.0 (7.4)        |

This pattern was significantly different than that observed in the RM group I, containing a higher percentage of lobules type 1 (p < 0.0008), whereas Lob 3 was significantly lower (p < 0.00004; Table 1).

The separation of the breast samples, based on the pregnancy history of the host, such as nulliparity and parity, indicated that in both subgroups, the percentage of Lob 1 was significantly higher than Lob 3 (Table 1) and that the differences between nulliparous and parous observed in the control or RM group were not present in the breast tissue derived from women with familial breast cancer (Table 1). Lob 1 represents 51.3% and 44.0% in the nulliparous and parous women, respectively. This indicates a reversion of the pattern observed in the parous in which Lob 1 is less frequent. In the familial cases, the comparison of the nulliparous from the RM group with those of the FAM group is not statistically different. Instead, the parous breast tissue of the RM group was significantly different than those of the FAM group (Table 3).

In order to determine whether those BRCA-1 positive (+) breast tissues with were different from those designated to be carriers, but in which BRCA status was not determined yet (Table 1), we separated these two groups (BRCA+ and carriers), and we found that the percentage of lobular structures was not significantly different.

The age of women from RM group was different from those of the FAM group; the average was 29.4 and 37.0 for the first group and second group, respectively. This difference is significant. In order to determine whether age may be contributory to the differences observed, we retabulated the data of the RM group for those women with matching age to those of the FAM group, and we found that the difference between both groups still persists, indicating that the familial factor could be in itself a deterrent in the pattern of architectural development of the breast.

The architectural pattern of the breast tissue obtained from MRM was from 43 breast samples. The average age for this group was 35.4 years, with no significant difference between the age for the nulliparous and parous women (Table 1). Quantitation of the three lobular structures in the overall population of breast tissue

Table 3. Profile of the Breast Tissue Obtained from Prophylactic Mastectomy in BRCA-1 Positive or Carrier of Familial Breast Cancer

| Sample number | Pedigree number | BRCA-1 | Age | Parity | Breast with cancer | Breast studied |
|---------------|----------------|-------|-----|--------|--------------------|----------------|
| 217           | 1973 – 11      | positive | 33  | yes    | right             | right and left |
| 231           | 3312 – 18      | carriers | 33  | no     | left              | left           |
| 234           | 1234 – 68      | positive | 33  | yes    | –                 | right and left |
| 222           | 3703 – 1       | carriers | 34  | no     | –                 | right and left |
| 221           | 3300 – 1       | carriers | 35  | yes    | –                 | right and left |
| 230           | 3619 – 5       | carriers | 36  | no     | –                 | right           |
| 205           | 2850 – 19      | carriers | 36  | no     | left              | right           |
| 203           | 3481 – 10      | carriers | 37  | yes    | right             | right and left |
| 202           | 3481 – 10      | carriers | 37  | yes    | right             | left            |
| 232           | 2887 – 3       | unlinked | 38  | no     | –                 | right & left    |
| 229           | 1816 – 62      | positive | 38  | no     | left              | left            |
| 206           | 1815 – 1       | carriers | 38  | yes    | right             | left            |
| 228           | 2552 – 13      | carriers | 39  | yes    | right             | left            |
| 210           | 1086 – 28      | carriers | 40  | yes    | right             | left            |
| 218           | 1816 – 680     | positive | 43  | no     | left              | right           |
| 223           | 1816 – 686     | positive | 37  | no     | right             | right and left  |
| 212           | 3386 – 1       | carriers | 43  | no     |                    | right and left  |
studied indicated that Lob 1 represented 74.25% of the structures, whereas Lob 2 and Lob 3 were 22.3% and 3.4%, respectively, of the total number of structures. The differences are statistically significant (Table 1).

The separation of the breast samples, based on the pregnancy history of the host, such as nulliparity and parity, showed no different pattern of lobular development. The breast of nulliparous women contained a significantly higher number of Lob 1 and Lob 2, 80.0% and 16.8%, respectively, and a highly significantly lower number of Lob 3 (1.7%; Table 1). In the breast of parous women, the pattern was similar, with Lob 1 and Lob 2 being the most abundant at 70.4% and 25.4%, respectively, whereas Lob 3 comprised only 3.8% of the total. The differences between nulliparous and parous were not statistically significant (Table 1).

The histological appearance of the lobular structures was not as different from that observed in the breast tissue of women who underwent RM, but it was when compared with the FAM group; 92.8% of the lobules were well defined in the RM group when compared with only 47.0% in the FAM group (3 out 43 breast samples were markedly fibrotic [Table 2], which was significantly lower than the FAM group, in which most of the lobules presented marked intralobular fibrosis). Ductal hyperplasia in Lob 1 or Lob 2, on the other hand, was observed in 62.9% of the cases and carcinoma in situ was observed in 11.4% of the cases (Table 4). Invasive carcinomas ipsilaterally or contralaterally were observed in 88.6% of the cases. The age of women from MRM group was not different from those of the FAM group (Table 1).

Altogether, these results show that the breast of parous women from the FAM and the MRM groups exhibited a different architectural pattern from those of parous women of the RM group, which can be considered the normal or control population (2.5). The observation that Lob 1 of the breast of both nulliparous and parous women of the FAM and MRM groups is the most frequent structure is in agreement with the knowledge that the cancer in the breast starts in Lob 1 (17,18,23). The greater proportion of Lob 1 found in the breast of nulliparous and parous women of the FAM and MRM groups suggests that these breasts were at higher risk of developing malignancies due to the fact that each Lob 1 is the target of carcinogenic insult (17,18).

**SPECIFIC CONSIDERATIONS ON THE RELATIONSHIP BETWEEN LOBULAR DEVELOPMENT AND FAMILIAL BREAST CANCER-RELATED GENES**

Currently, we do not know which is the gene(s) that is controlling the differentiating pattern of the human breast (23,61). It has been postulated that BRCA-1 (61) and/or BRCA-2 may serve to control cell proliferation and differentiation during developmental stages characterized by rapid growth (62). This model predicts that individuals possessing germline mutations in BRCA-1 and/or BRCA-2 may be particularly susceptible to early events in mammary carcinogenesis during pregnancy (62). Recent epidemiological observations suggest that women with a positive family history of breast cancer may experience a significantly greater increase in breast cancer risk associated with their first pregnancy relative to women without a family history of breast cancer (63). How BRCA-1 and/or BRCA-2 control breast differentiation is unknown. In this work, we indicate that in both sporadic and familial breast cancer, the pattern of lobular development is very similar. In rodents as well as in the breast tissue of women who underwent plastic surgery for cosmetic reasons, parity is associated with lobular differentiation (10,23). In both cases, lobular differentiation makes the mammary tissue refractory to neoplastic transformation by chemical carcinogens (64). Moreover, the relationship of the differentiation effect induced by pregnancy and the induced protection against breast cancer in women who have undergone this FFTP early in life (65) are indications that the same operational events are modified in both familial and sporadic cases of breast cancer.

In addition to the overall architectural differences described previously in this article, the breast tissues from women with hereditary breast cancer present histological differences in the intralobular stroma. The intralobular stroma, different from the more dense collagenized interlobular stroma, is a dynamic compartment of the
breast composed of loosely arranged connective tissue containing cells such as fibroblasts, blood vessels, and inflammatory cells such as lymphocytes, mast cells, and macrophages (66,67). The intralobular stroma contrasts with the interlobular stroma, which has fewer cells separated by larger quantities of more compact collagen. The role of intralobular stroma during breast development from adolescence to premenopausal maturity, pregnancy and lactation, involution, and postmenopausal changes has been implicated; however, how the interaction with the epithelial cells takes place is unknown. Most of our understanding of the interaction between epithelial and stroma in the breast is from the experiments of Sakakura (68). In this work, we indicate that the intralobular stroma of the Lob 1 of the breast of women with familial breast cancer has lost the loosely arranged connective tissue for a more dense stroma that erases its demarcation from the intralobular stroma. The intralobular stroma of the breast tissue from the FAM group was more fibrotic and dense. These findings suggest that in the breast cancer families either the development of the breast parenchyma has failed to respond to the normal physiological stimuli that determine the formation of lobular structures, indicative of differentiation, or the involution pattern of the lobular structures type 3 after pregnancy is more rapid in these women than in those in the control. It is noteworthy that early pregnancies influence breast cancer risk by altering the structure of the mammary parenchyma (17,25). It has been hypothesized that late pregnancies could likewise influence breast cancer risk via alterations in the mammary parenchyma by delaying or interrupting the normal process of involution of glandular tissue of the breast (69). In the mouse, it has been observed that BRCA-1 is induced during puberty, pregnancy, and following treatment of ovariectomized animals with 17-β-estradiol and progesterone (70). Therefore, is not surprising that in the human breast, alteration of this gene may explain the altered morphological pattern observed. Our findings that the intralobular stroma is more fibrotic in the FAM group than in the MRM and RM groups may explain in part the increased mammographic density in women with familial breast cancer (71–74). Although the intralobular stroma is only a small component of all of the factors that determine the mammographic pattern, the mammographic breast density reflects proliferation of breast stroma through collagen formation and fibrosis (75). The factors that determine breast densities depend on the interplay of hormones, such as estrogen and growth factors, such as epidermal growth factor, transforming growth factor, and insulin growth factors I and II (76). How all of these factors and the genes related to familial breast cancer interrelate in the biology of the intralobular stroma is not known.

The development of mammary ductal structures involves a complex interplay between epithelium and mesenchyme (68,77–81), and the branching of the mammary ducts is dependent on circulating hormones for stimulation and synchronization with reproductive events but is also influenced by local factors to provide signals that influence glandular growth, differentiation, and morphogenesis. The matrix-degrading metalloproteinase stromelysin-1, stromelysin-3, and gelatinase A are expressed during ductal branching morphogenesis of the murine mammary gland (82). Whereas the role of metalloproteinases in the branching pattern of the mammary gland and its relationship with BRCA-1 requires further investigation, on the basis of these data it is possible to postulate that the breast tissue from women with hereditary breast cancer suffers from an alteration of the interaction between the epithelium and the stroma, resulting in a modified interaction between the stroma parenchyma as described here.

Our work also confirmed the previous observation reported in the literature (83) that breast tissue of women from sporadic breast cancer contained higher numbers of ductal carcinoma in situ and ductal hyperplasias than those from the familial breast cancer. Overall, our data indicate that BRCA-1 or related genes associated with familial breast cancer play a role in the lobular pattern of the breast mainly by altering the pattern of involution after pregnancy, with a consistent increase in the Lob 1 as compared with the control population. However, the fact that familial breast. We concluded that the developmental pattern of the breast of parous women of the familial breast cancer group was similar to that of nulliparous women of the same group and was less developed than the breast of parous women without history of familial breast cancer. The breasts of women belonging to the familial breast cancer group also presented differences in the branching pattern of the ductal epithelium, observations that suggested that the genes that control lobular development might have been affected in those women belonging to families with a history of breast and breast/ovarian cancer (19). Supporting evidence to this fact is the poor milk production reported in carriers of the BRCA-1 mutation compared with female relatives without mutation (84) and the poor
differentiation of the mammary gland of mice with BRCA-1 mutations (85).

**UNIFYING CONCEPTS**

Breast cancer originates in undifferentiated terminal structures of the mammary gland. The terminal ducts of the Lob 1 of the human female breast, which are the sites of origin of ductal carcinomas, are at their peak of cell replication during early adulthood, a period during which the breast is more susceptible to carcinogenesis. The susceptibility of Lob 1 to undergo neoplastic transformation has been confirmed by in vitro studies, which have shown that this structure has the highest proliferative activity and rate of carcinogen binding to the DNA. More importantly, when treated with carcinogens in vitro, its epithelial cells express phenotypes that are indicative of cell transformation (19,22). These studies indicate that in the human breast, the target cell of carcinogens is found in a specific compartment in which the characteristics are the determinant factors in the initiation event. These target cells will become the stem cells of the neoplastic event, depending on (a) topographic location within the mammary gland tree, (b) age at exposure to a known or putative genotoxic agent, and (c) reproductive history of the host. The higher incidence of breast cancer observed in nulliparous women supports this concept because it parallels the higher cancer incidence elicited by carcinogens in rodents when exposure occurs at a young age.

In addition, it has been shown that an increase in parity is associated with a pronounced decrease in the risk of breast cancer, each additional live birth conferring a 10% risk reduction(6). Thus, the protection afforded by early full-term pregnancy in women could be explained by the higher degree of differentiation of the mammary gland at the time in which an etiologic agent or agents act. Even though differentiation significantly reduces cell proliferation in the mammary gland, the mammary epithelium remains capable of responding with proliferation to given stimuli, such as a new pregnancy. Under these circumstances, however, the cells that are stimulated to proliferate are from structures that have already been primed by the first cycle of differentiation, thus creating a second type of stem cell that is able to metabolize the carcinogen and repair the DNA damage induced more efficiently than the cells of the virginal gland and is less susceptible to carcinogenesis, as it has been demonstrated in the rodent experimental system(55). However, a carcinogenic stimulus powerful enough may overburden the system, successfully initiating a neoplastic process. These conditions might explain the small fraction of women developing breast cancer after an early FFTP, meaning completion of the first cycle of differentiation. The relevance of our work lies in the vis-à-vis comparison of in vivo and in vitro studies in the human breast that validates experimental data for extrapolation to the human situation. The finding that differentiation is a powerful inhibitor of cancer initiation provides a rationale in identifying the genes controlling this process. The knowledge gained will provide novel tools for developing rational strategies for breast cancer prevention.

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