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DIFFERENTIATION AND BREAST CANCER

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Summary The mammary gland is an organ whose size, shape and function undergo fundamental changes during the various phases of a woman's growth. Although the development of the mammary gland begins during infancy, the most dramatic changes occur with the initiation of puberty. Pregnancy and lactation complete the functional development of the organ, which regresses during menopause. Epidemiological and experimental studies have demonstrated that certain hormonal influences, especially those related to reproduction, modify the risk of developing breast cancer. Thus, a full term pregnancy completed before the age of 24 years significantly reduces the lifetime incidence of breast cancer. Although the mechanism through which pregnancy protects the breast from breast cancer has not been clearly established, experimental models of mammary carcinogenesis have allowed researchers to determine that pregnancy inhibits the initiation of the neoplastic process through the induction of a complete differentiation of the mammary gland. This process activates specific genes, which in turn modify the response of the organ to ulterior hormonal changes. It is postulated that the same mechanism might be responsible for the protective effect of a woman's early first full term pregnancy. The greater incidence of breast cancer observed in nulliparous women correlates well with the greater susceptibility of the virgin rat to develop mammary carcinomas when exposed to chemical carcinogens. The successful induction of malignant transformation in the virgin animal mammary epithelium is due to the presence of undifferentiated structures with a high rate of cell proliferation. These structures are eliminated by pregnancy. The breast of nulliparous women retains those undifferentiated structures, which increase the predisposition of the organ to undergo malignant transformation, which will manifest itself clinically several years after its initiation. The correlation of human epidemiological, clinical and experimental data with those data obtained in rodent experimental models lends support to this hypothesis.

Key words: breast cancer, cellular differentiation

The breast presents dramatic changes in size, shape and function during growth, puberty, pregnancy, lactation and post-menopausal regression.[1-4] The fact that the breast is the source of the most frequent malignancy in the female population, and the knowledge that breast cancer is heavily influenced by the reproductive history of the individual,[1-3] requires a thorough understanding of how pregnancy influences the development of this organ. Although the development of the human breast starts during embryonic life, the main spurt of growth with lobule formation occurs at puberty, but breast development and differentiation are completed only at the end of a full term pregnancy.[1,2] It has long been known that the risk of breast cancer shows an inverse relationship with early parity.[1-9] Case control studies have demonstrated that breast cancer risk increases

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with the age at which a woman bears her first child; the important factor in this protection seems to be related to the interval of time between menarche and the first pregnancy, since increased risk has been reported when this interval is lengthened over 14 years.[9] Thus, to be protective, pregnancy has to occur before age 30 –indeed women first becoming pregnant after that age appear to have a risk above that of nulliparous women.[9] Although multiparity appears to confer additional protection, the protective effect remains largely limited to the first birth. The protection conveyed by an early reproductive event persists at all subsequent ages, even until women become older than 75 years of age.[4, 9] Although the ultimate mechanisms through which an early first full term pregnancy protects the breast from cancer development are not known, a likely explanation has been provided by studies performed in an experimental animal model. Induction of mammary carcinomas with chemical carcinogens in rats has revealed that full term pregnancy inhibits carcinogenic initiation through the induction of differentiation. It can be postulated that gland differentiation activates specific genes that imprint the breast epithelia to subsequent hormonal milieu, and that this is also responsible for the protection that an early full term pregnancy confers to women. There is no explanation for the higher risk to develop malignancies exhibited by nulliparous and late parous 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,[2, 10-15] suggests that the breast of late parous and of nulliparous women might exhibit some of the undifferentiated and/or cell proliferative characteristics that predispose the tissue to undergo neoplastic transformation. The correlation of our findings in the experimental animal model with those obtained through the study of the development of the human breast, support this postulate.

Hormones and growth factors on breast development

The reproductive process, since its initiation, is deeply dependent of hormonal and neural factors. The maternal corpus luteum, that is instrumental in the preparation of the endometrium for implantation, is in turn, rescued by the luteotropic hormone chorionic gonadotropin (CG) secreted by the primitive trophoblast of the blastocyst within hours of implantation. In women, human CG (hCG) stimulates the corpus luteum to synthesize progesterone, 17-hydroxyprogesterone, estradiol, inhibin, and relaxin. The corpus luteum constitutes the major source of progestational steroids until the ninth week of gestation, when the placenta becomes the sole source of these hormones, as demonstrated by the lack of effect of ovariectomy after the ninth week on the progression of pregnancy. The placenta has evolved in mammals as an efficient mechanism for transporting nutrients to the fetus, excreting waste products into the maternal blood stream, and for influencing maternal physiology through the newly secreted placental and fetal hormones. In humans, the placenta becomes fully developed by the end of the first trimester of pregnancy. The syncytiotrophoblast of the chorionic villus, the functional unit of the placenta, synthesizes progesterone, hCG, and human placental lactogen (hPL). The cytotrophoblast is the source of several neuropeptides first discovered in the brain, such as gonadotropin releasing hormone (GnRH), thyrotropin releasing hormone (TRH), somastotatin, corticotrophin releasing factor (CRF), and propiomelanocortin, and the gonadal peptide inhibin.[16-22] Chorionic gonadotropin (CG) is a polypeptide hormone composed of an α and β subunits. The α subunit is identical to that of pituitary gonadotropins, whereas the β subunit differs in aminoacid sequence.[23] The most widely known action of CG is the maintenance of the corpus luteum during pregnancy, an action that is identical to that of the pituitary gonadotropin LH, with a small degree of FSH activity.[24, 25] The breast has been traditionally considered to be a passive target of sex steroid hormones. This concept, however, is being challenged by modern day research reporting the discovery of a direct inhibitory effect of hCG on human breast epithelial cell proliferation, and the induction of inhibin synthesis.[24] New hormones and growth factors are being discovered to be locally synthesized in the breast, suggesting that this organ might self-regulate its development through autocrine or paracrine effects. This promissory area of research remains to be developed.

The human breast as a developing organ

The human breast is one of the few organs of the body that is not completely developed at birth; it reaches its fully differentiated condition only after 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 fully secretory lobular structures.[10] The study of the branching pattern of the breast requires a tridimensional analysis of the organ in order to evaluate the relationship of terminal and lateral branches to the main lactiferous ducts. A more limited vision is provided by the classical two-dimensional histological sections in which the topographic arrangement of ducts, ductules and alveoli or acini allows one to reconstruct and categorize specific lobular units. Each lobular structure has been morphologically characterized by their size, number of ductules per unit, and the number of cells per ductule, reflecting different stages of development. The earliest or more undifferentiated structure identified in the breast of postpubertal nulliparous women is the lobule type 1 (Lob 1), also called terminal ductal lobular unit (TDLU); it is composed of clusters of 6 to 11 ductules per lobule. They progress to lobules type 2 (Lob 2), which have a more complex morphology, being composed of a higher number of ductular structures per lobule. During pregnancy, Lob 1 and Lob 2 rapidly progress to lobules type 3 (Lob 3), and secretory lobules type 4 (Lob 4). Lob 3 are characterized by having an average of 80 small alveoli per lobule (Figure 1). When active milk secretion supervenes, the alveoli become distended, a characteristic of the Lob 4 present during the lactational period. After weaning, all the secretory units of the breast regress, reverting to Lob 3 and Lob 2.

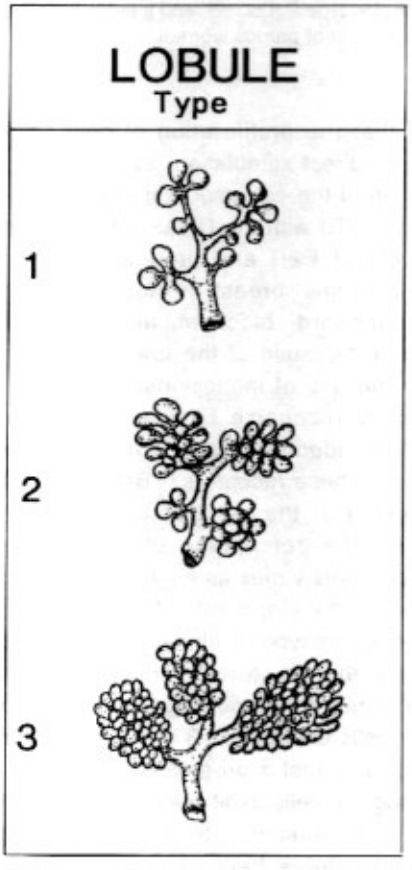

Fig. 1.– Schematic representation of the lobular structures in the human breast.

Cell proliferation and hormone receptors in relation to breast structure

Although ductal breast cancer originates in Lob 1, or TDLU,[11] the epidemiological observation that nulliparous women exhibit a higher incidence of breast cancer than early parous women,[4] suggests that Lob 1 in these two groups of women might be biologically different, or exhibit different susceptibility to carcinogenesis.[13, 26-28] At the present time it is not known whether specific genes are responsible of or control these differences. What is known is that the branching of the mammary ducts proceeds under the influence of circulating hormones for stimulation and synchronization with reproductive events. It is also influenced by local factors which provide signals that influence glandular growth, differentiation and morphogenesis. Cell proliferation is a cell function essential for normal growth. It also plays a crucial role in the development of malignancies.[1, 14, 15] Normal growth requires a net increase of cycling cells over two other cell populations, resting cells (arrested in G_0, and dying cells (cells lost through programmed cell death or apoptosis). The proliferative activity of the mammary epithelium varies as a function of the degree of lobular differentiation. Lob 1 have a higher proliferative in-

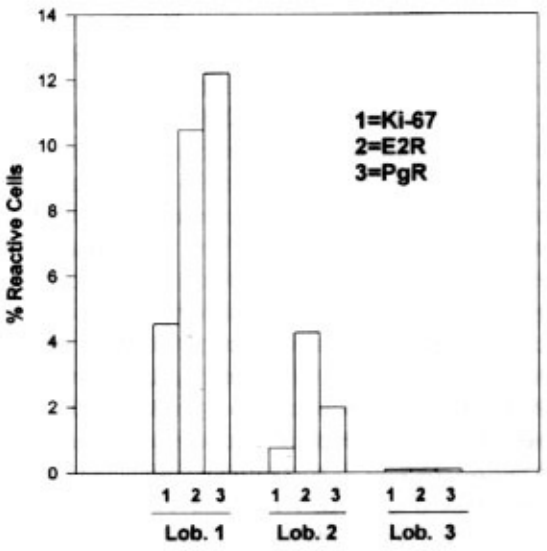

Fig. 2.– Proliferative activity determined by Ki67, estrogen receptors (E2R) and progesterone receptors (PgR) detected immunocytochemically and expressed as the percentage of positive cells in the lobules type 1 (Lob. 1), lobules type 2 (Lob. 2), and lobules type 3 (Lob. 3) of the breast of nulliparous women.

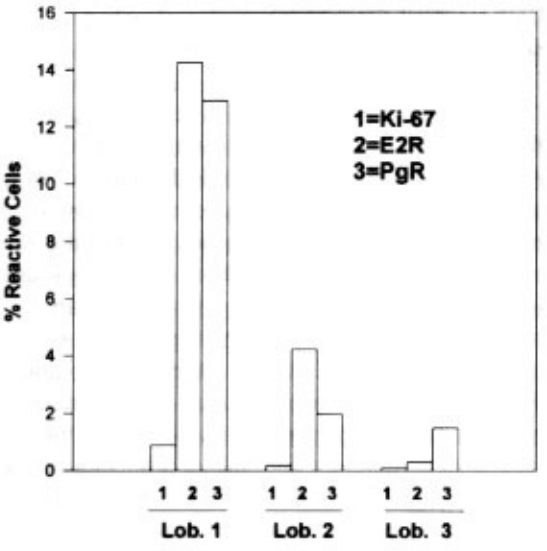

Fig. 3.– Proliferative activity determined by Ki67, estrogen receptor (E2R) and progesterone receptors (PgR) detected immunocytochemically and expressed as the percentage of positive cells in the lobules type 1 (Lob. 1), lobules type 2 (Lob. 2), and lobules type 3 (Lob. 3) of the breast of parous women.

dex than Lob 2, 3 and 4 (Figure 2). These differences are not abrogated when the phases of the menstrual cycle are taken into consideration.[29] Parity, in addition of exerting an important influence in the lobular composition of the breast, as described above, profoundly influences the proliferative activity of the mammary epithelium. Lob 1 and Lob 2 present in the breast of premenopausal nulliparous women exhibit a significantly higher proliferative activity than those lobules found in the breast of parous women (Figure 3). After menopause sets in the proliferative activity of the mammary epithelium decreases, but although less pronounced, the differences between the nulliparous woman and parous women's cell proliferation in breast structures are maintained.

Estrogens and progesterone are known to promote proliferation and differentiation in the normal breast epithelium. Both steroids act intracellularly through a receptor which, when activated by its binding with the hormone, regulates the expression of specific genes.[30, 31] However, the mechanism by which these molecules exert their mitogenic and differentiation effect has not been clearly established.[32-40] One of the accepted mechanisms of action of steroid hormones postulates that the proliferation of cells is the response to direct stimulation, as the result of the interaction of the estradiol bound to the estrogen receptor (E2R) with the DNA.[31] Measurements of the levels of E2R and progesterone receptor (PgR) in normal breast in the cytosol fraction, using standard biochemical techniques, is inaccurate because of the low cellularity of the tissue. The use of monoclonal antibodies which specifically recognize E2R and PgR makes it possible to identify and to quantitate the cells expressing these receptors.[29] Both E2R and PgR are present in the nucleus of epithelial cells. However, 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 are the structures more consistently containing a higher percentage of E2R and PgR positive cells than Lob 2, 3 and 4, an observation that indicates that a progressive decrease in the percentage of cells exhibiting an immunocytochemically positive reaction for these markers occurs as the structures become more differentiated (Figures 2 and 3). These data allowed us to conclude that the degree of differentiation of the

breast is an important determinant in the expression of both E2R and PgR, in addition to modulate the proliferative activity of the breast epithelium. Neither age nor parity (Figures 2 and 3) history affect the percentage of cells reacting for both receptors.

The Lob 1 as the site of origin of breast cancer

An important concept that emerged from our study of breast development is that the TDLU, which had been originally identified by Wellings, et al.[41] as the site of origin of the most common breast malignancy, the ductal carcinoma,[41, 42] corresponds to a specific stage of development of the mammary parenchyma, the lobule type 1 (Fig. 4). This observation is supported by comparative studies of normal and cancer-bearing breasts obtained at autopsy. It was found that the non tumoral parenchyma in cancer associated breasts contained a significantly higher number of hyperplastic terminal ducts, atypical Lob 1 and ductal carcinomas *in situ* originated in Lob 1 than those breasts of women free of breast cancer. These observations indicate that the Lob 1 is affected by preneoplastic as well as by neoplastic processes.[14, 15] The finding that the most undifferentiated structures originate the most undifferentiated and aggressive neoplasms acquires relevance to the light that these structures are more numerous in the breast of nulliparous women, who are, in turn, at a higher risk of developing breast cancer. We concluded that the Lob 1 found in the breast of nulliparous women never went through the process of differentiation, whereas the same structures, when found in the breast of postmenopausal parous women did.[14] More differentiated lobular structures have been found to be affected by neoplastic lesions as well, although they originate tumors whose malignancy is inversely related to the degree of differentiation of the parent structure, ie., Lob 2 originate lobular carcinomas *in situ*, whereas Lob 3 give rise to more benign breast lesions, such as hyperplastic lobules, cysts, fibroadenomas and adenomas, and Lob 4 to lactating adenomas[11] (Figure 4). We concluded from these observations that each specific compartment of the breast gives origin to a specific type of lesion. The finding that the most undifferentiated structures originate the most undifferentiated and aggressive neoplasms acquires relevance to the light that these structures are more numerous in the breast of nulliparous women, who are, in turn, at a higher risk of developing breast cancer.

Transformation of human breast epithelial cells

It is not known when in the lifetime of a woman the initiation of breast cancer takes place, or whether a specific agent causes it. The facts that both late menarche and a full-term pregnancy completed before age 24, or early full-term pregnancy, reduce the risk of breast cancer development, whereas early menarche, nulliparity and exposure to ionizing radiations at ages younger than 19 are associated with a higher breast cancer incidence,[5, 11] indicate that the period encompassed between menarche and first full-term pregnancy represents a window of high susceptibility for the initiation of breast cancer. In the previous section it has been indicated that ductal carcinomas originate in TDLU (Lob 1) and lobular carcinomas in Lob 2, whereas the Lob 3 is not associated with the development of malignancies.[11, 41] In order to ascertain whether Lob 1 and Lob 2 are more susceptible than Lob 3 to undergo neoplastic transformation, we have

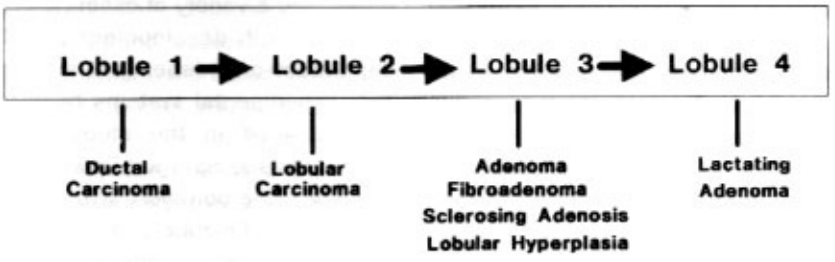

Fig. 4.- Schematic representation of the pathogenetic pathway of breast cancer. Reproduced from Russo and Russo[26], with permission.

developed an *in vitro* system that reproduces the *in vivo* conditions of the breast epithelium. For these purposes, we utilize normal breast tissues from reduction mammoplasties which are obtained fresh and sterile. Upon digestion of the tissues with collagenase and hyaluronidase, epithelial cells in aggregates, or organoids, are separated by micromanipulation. Organoids are classified as Lob 1, Lob 2 or Lob 3 by applying the same criteria developed for classifying these structures in whole mount and histopathological preparations. Plating of each lobular type separately allows one to evaluate whether the behavior of cells in culture correlates with the specific type of lobule that originated them. Cells from Lob 1 and Lob 2, which in organ culture have shown to exhibit a higher DNA-LI, attach to the dishes promptly and start growing logarithmically, whereas cells from Lob 3, which have a lower DNA-LI, have a long lag phase before they attach to the dish and start growing. We determined that the number of doublings per unit of time was also higher in Lob 1 and Lob 2 than in Lob 3.[13] For testing the susceptibility of the different lobule types to be transformed by chemical carcinogens *in vitro* a total of 52 human breast samples were processed. Organoids representing Lob 1, Lob 2 and Lob 3 were plated, and when the cells reached their logarithmic phase of growth they were treated with the chemical carcinogens N-methyl-N-nitrosourea (NMU), 7,12-dimethyl-benz(a) anthracene (DMBA), methyl-N-nitro-nitroso-guanidine (MNNG) or benz(a)pyrene(BP) for 24 hours. The cells were followed up for several passages until they exhibited changes indicative of neoplastic transformation, such as variations in cell morphology, loss of contact inhibition, and anchorage independent growth. The changes in cell shape induced by the carcinogens was the result of increased number of surface microvilli and decreased cell-cell interaction. The property to form domes when plated in plastic flasks, which is characteristic of normal breast epithelial cells, was lost in carcinogen treated cells; this phenomenon was interpreted to be the result of an abnormal pattern of growth caused by altered contact inhibition. Treated cells showed increased ability to survive and to form colonies in agar methocel, and to exhibit multinucleation.[26] These types of responses, however, were observed only in the epithelial cells derived from breast tissues containing Lob 1 and Lob 2. The phenomena were not observed in the breast cells derived from Lob 3.[26]

These observations led us to conclude that primary cultures of human breast epithelial cells are susceptible to be transformed *in vitro* by chemical carcinogenes, however, the expression of phenotypes indicative of neoplastic transformation depends upon the stage of development of the breast and of the *in vivo* cell proliferation rate.[26] The finding that Lob 1 and Lob 2 express more readily changes indicative of neoplastic transformation *in vitro* indicates that these structures are more susceptible to the transforming effect of genotoxic agents, thus supporting the observations that they are the site of origin of mammary carcinomas; it also correlates with the lack of association of the Lob 3 with the development of malignant neoplasms.[12, 26] Of greater relevance is the observation that the breast of nulliparous women contains more numerous Lob 1 and Lob 2 than the breast of parous women, in which predominates the Lob 3, further emphasizing the protective effect of gland differentiation, which modulates the response of breast epithelial cells to carcinogens under *in vitro* conditions.

Experimentally induced mammary tumors in rats

The elucidation of how host factors influence the initiation of the neoplastic process, and the determination of whether in women the susceptibility of the mammary gland varies with age and reproductive history require the availability of adequate experimental models. The induction of rat mammary carcinomas with chemical carcinogens, one of the most widely studied models more closely fulfills the above requirements. Many strains of rats develop spontaneous tumors, and respond to a variety of chemical carcinogens and radiation with development of either hormone-dependent or independent mammary tumors.[11] Two experimental systems have been preferentially utilized in the study of rat mammary tumorigenesis, Sprague-Dawley (S-D) rat inoculation with the polycyclic hydrocarbon DMBA,[43, 44] and S-D or Fischer 344 rat injection of NMU. DMBA, given by gavage in a single dose of 2.5 to 20 mg induces tumors with latencies that gen-

erally range between 8 and 21 weeks, with final tumor incidences close to 100% if sufficient time elapses before necropsy. NMU, given by intravenous or subcutaneous injection in a single dose of 25 or 50 mg/kg body weight yields tumors with latency and incidence similar to those reported for DMBA.[11, 45]

These models of mammary carcinogenesis constitute useful tools for analyzing the interaction of the two major basic components of the neoplastic process, the etiologic agent, in this case the chemical carcinogen, and the target organ, obviously the mammary gland.[46] The mammary gland, however, does not respond as a unit to the carcinogenic insult. The mammary parenchyma develops from the superficial ectoderm as a complex tubular branching system that invades the stroma through active growth centers, the terminal end buds (TEBs).[47, 48] The TEB is a primitive element of the mammary parenchyma; at the time of pubertal growth in the rat (at about 25-35 days of age) it starts to bifurcate into alveolar buds (ABs); these, with successive estrous cycles progress to virginal lobules. The administration of DMBA to virgin rats elicits a tumorigenic response whose incidence is directly proportional to the density of TEBs primed by the ovarian hormones for their differentiation to ABs.[1, 47, 48] This postulate is supported by the observations that although 100% incidence of carcinomas is elicited when DMBA is administered to rats between the ages of 30 and 55 days, the highest number of tumors per animal develops when the carcinogen is given to animals when they are 40 to 46 days of age, a period when TEBs are most actively differentiating into ABs. The sharp decrease in the number of TEBs observed in animals older than 55 days is accompanied by a lower incidence of tumors, as well as a lower number of tumors per animal[1, 47, 48].

The first lesions induced by DMBA consist in the enlargement of one or various adjacent TEBs, which appear darkly stained in whole mount preparations. These lesions, observed between 7 and 14 days post-treatment, are called intraductal proliferations (IDPs).[1] Lesions arising in adjacent TEBs tend to coalesce forming microtumors, which becore evident after 20 days of DMBA administration.[46] IDPs are lined by a multilayered epithelium; when they progress to intraductal carcinomas they exhibit a marked stromal reaction with desmoplasia, and infiltration by mast cells and lymphocytes. From these structures the fully developed or palpable tumors grow into invasive carcinomas with cribriform, comedo or papillary patterns. Some tumors metastasize, mainly to the lungs, if the animals are allowed to live long enough. DMBA induces an array of benign lesions, such as cysts, adenomas, alveolar hyperplasias and fibroadenomas, which are originated from more differentiated structures, such as ABs and virginal lobules. They appear much later than IDPs and intraductal carcinomas. These observations indicate that there are two different pathogenetic pathways, one for the malignant and another for the benign lesions. The fact that benign lesions appear later than the malignant ones indicate that the former are not precursors of the latter.[1, 11, 47, 48]

A further evidence in support of the TEB as the site of origin of mammary carcinomas has been obtained by plotting the incidence of adenocarcinomas against the percentage of TEBs, ABs and lobules present in the mammary gland at the time of carcinogen administration. A high correlation coefficient has been found between the incidence of carcinomas and the number of TEBs, but not between tumor incidence and the number of the other terminal structures.[47] Further evidence that it is the number of TEBs which affects the susceptibility of the mammary gland to carcinogenesis has been obtained from the study of the influence of pregnancy on mammary cancer initiation. Full term pregnancy, which completely eliminates the TEBs in the mammary gland through the induction of full differentiation, if completed prior to carcinogen administration, inhibits tumor development.[1, 47-49]

If pregnancy is interrupted, however, this protection is minimized or nullified.[49, 50] Hormonal treatment with estrogenic compounds[51-53] or with chorionic gonadotropin (hCG)[54-62] also reduces the number of TEBs through the induction of changes similar to those occuring with pregnancy. The degree of differentiation induced by these hormonal treatments correlates with the degree of refractoriness to undergo malignant transformation. One of the elements that influence the susceptibility of the TEB to carcinogenesis is the high proliferative activity of its epithelium. Determination of the mammary gland's growth fraction has

revealed that the largest compartment is in the TEBs, in which 55% of the cells are proliferating, whereas in ABs and lobules only 23% of the cells are in the proliferative pool. The growth fraction decreases with both aging and differentation. In the mammary gland of parous animals the growth fraction is only 1%.[54-63]

Carcinogenic initiation requires the stable alteration of DNA molecules, a process that requires that the carcinogen binds to the DNA.[64-66] Maximal DNA binding occurs during DNA synthesis, thus, carcinogens damage DNA mostly during the S-phase.[67, 68] If the damage is not repaired during the G1 phase, this damage is transmitted to the daughter cells and it becomes fixed during successive S-phases of the cycle. We have demonstrated that the uptake of ^{3}H-DMBA is selectively higher in TEBs than in other structures of the mammary gland; this uptake, expressed as the number of grains per nucleus, highly correlates with the DNA synthetic activity of the cells or DNA-LI.[1] DMBA is metabolized by the mammary epithelium to both polar and phenolic metabolites. The metabolic pathway is similar in both the TEBs of virgin rats and the lobules of parous animals; however, the formation of polar metabolites is higher in the epithelial cells of TEBs, in which the binding of the carcinogen to DNA is also higher.[11] Removal of adducts from the DNA differs between TEBs and lobules; the former have a very low rate of adduct removal, whereas the latter are more efficient, indicating that the lobules repair the damage induced by the carcinogen more efficiently.[10]

These data allowed us to conclude that the susceptibility of the mammary gland to carcinogenesis is modulated by the following parameters: 1) the presence of terminal end buds; 2) the size of the proliferative compartment; 3) the amount of binding of the carcinogen to the DNA, and 4) the ability of the cells to repair the DNA damaged by the carcinogen.

Unified concept of mammary carcinogenesis

Comparative studies between humans and rodents have allowed us to determine that mammary cancer originates in undifferentiated terminal structures of the mammary gland. The terminal ducts of the Lob 1 or TDLU of the human female breast have many points in common with the TEB of the rat mammary gland. Firstly, the TEB in the rat and the Lob 1 in women are both the site of origin of ductal carcinomas. Secondly, cell replication in Lob 1 is at its peak during early adulthood, at a time during which the breast is more susceptible to carcinogenesis, decreasing considerably with aging. TEBs have also their highest proliferative activity when the animals are young, a period of greater susceptibility to undergo malignant transformation due to the greater binding of carcinogen to the DNA, and lower cell repair; aging results in decreased proliferative activity and susceptibility to carcinogens. The parallelism we have found between the TEB, that when affected by DMBA evolves to IDPs, carcinoma *in situ*, and invasive carcinoma, and the Lob 1, the site of origin of mammary carcinomas, has been further confirmed by in vitro studies, that have confirmed that like TEBs, Lob 1 have also the highest proliferative activity and greater carcinogen binding to the DNA; more importantly, when treated with carcinogens *in vitro* they express phenotypes indicative of cell transformation. These comparative studies indicate that both in rodents and in humans there is a target cell of carcinogenesis, which is found in a specific compartment whose characteristics are a determinant factor in the initiation event (Figure 5). These target cells will become the "stem cells" of the neoplastic event, depending upon: a) topographic location within the mammary gland tree, b) age at exposure to a known or putative genotoxic

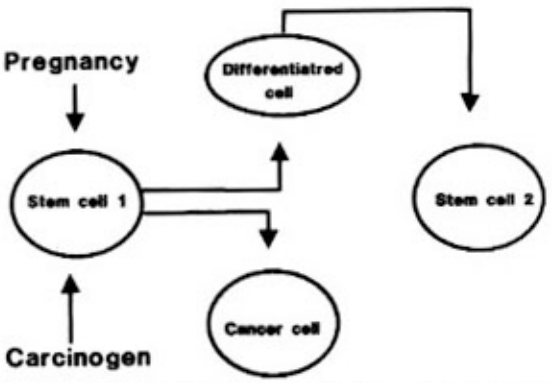

Fig. 5.– Evolution of the Stem cell 1 of the mammary gland under the effect of a carcinogen or the physiological stimulus of pregnancy. Reproduced from Russo, J. and Russo, I.H. Endocrine Related Cancer 4:1-15, 1997, with permission.

agent, and c) reproductive history of the host. Epidemiologic findings, such as the higher incidence of breast cancer in nulliparous women and in women having an early menarche support this concept, since it parallels the higher cancer incidence elicited by carcinogens when exposure occurs at a young age, and in nulliparous animals. In both cases, the mammary tissue contains predominantly undifferentiated structures. Thus, the protection afforded by early full term pregnancy in women, or full term pregnancy or hormonal treatment in rodents 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, nevertheless, 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 cells" that are able to metabolize the carcinogen and repair the DNA damage induced more efficiently than the cells of the virginal gland, and are, therefore, less susceptible to carcinogenesis (Figure 5). A carcinogenic stimulus powerful enough may overburden the system, successfully initiating a neoplastic process. These conditions might explain the small fraction of tumors developing in the mammary gland exposed to a carcinogenic stimulus after completion of the first cycle of differentiation. The relevance of our work lies in the side to side comparison of findings in an experimental animal model and in the human breast, that validates experimental data for extrapolation to the human situation. The findings that cell proliferation is of important for cancer initiation, whereas differentiation is a powerful inhibitor provide novel tools for developing. rational strategies for breast cancer prevention.

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Resumen

Diferenciación y cáncer de mama

La glándula mamaria es un órgano cuyo tamaño, forma y funcionalidad cambian fundamentalmente durante las diferentes fases de crecimiento de la mujer. Aunque el desarrollo de la glándula mamaria comienza durante la vida embrionaria y continúa durante la infancia, los cambios más dramáticos ocurren con la iniciación de la pubertad. El embarazo y la lactancia completan el desarrollo funcional del órgano, el cual regresa durante la menopausia. Estudios epidemiológicos y experimentales han demostrado que ciertas influencias hormonales, especialmente aquéllas relacionadas con la reproducción, modifican el riesgo de desarrollar cáncer de mama. Por ejemplo el embarazo completado antes de los 24 años de edad reduce significativamente la incidencia de cáncer de mama por el resto de la vida de la mujer. Aunque el mecanismo por el cual el embarazo disminuye el riesgo de desarrollar cáncer de mama no está claramente establecido, los modelos experimentales de carcinogénesis mamaria indican que la preñez inhibe la iniciación del proceso neoplásico a través de la inducción de una diferenciación completa de la glándula. Este proceso activa genes específicos que modifican la respuesta del órgano a cambios hormonales ulteriores. Se postula que este mismo mecanismo puede ser el responsable de la protección conferida a la mujer por una preñez temprana. La mayor incidencia de cáncer en la mujer nulípara se correlaciona con la mayor susceptibilidad de la rata virgen a desarrollar carcinomas mamarios cuando se le administran carcinógenos químicos. A fin de inducir con éxito la transformación maligna del epitelio mamario se requiere que el animal sea virgen y que su glándula mamaria contenga estructuras indiferenciadas con un alto nivel de proliferación celular. Estas estructuras son eliminadas por el embarazo a término. La mama de la mujer nulípara retiene estas estructuras indiferenciadas, las cuales aumentan la predisposición de este órgano a sufrir una transformación maligna, la cual se manifiesta clínicamente varios años después de su iniciación. La correlación de los datos clínicos y experimentales en humanos con los datos obtenidos en modelos experimentales en roedores dan apoyo a esta hipótesis.

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