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A NEW PARADIGM IN BREAST CANCER PREVENTION

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REVIEW ◇ HYPOTHESIS

ABSTRACT. THE INCURABILITY of breast cancer, in association with a world-wide increase in its incidence, indicates that primary prevention is the ultimate goal for breast cancer control. It is in this stage of knowledge that we have developed a new paradigm for breast cancer prevention. Our paradigm has emerged from epidemiological observations of a direct association of breast cancer risk with nulliparity and of protection conferred by an early first full term pregnancy. We have chosen this specific strategy because it is a window of opportunity that nature has provided us for learning how a physiological event produces in a significant percentage of women a complete protection against cancer. Our studies have unraveled the biological principle underlying the protection conferred by an early first full-term pregnancy. Furthermore, we have also demonstrated that the same degree of protection can be elicited by a short treatment with recombinant human chorionic gonadotropin (r-hCG), a hormone secreted during pregnancy. Both pregnancy and r-hCG treatment induce in the breast the expression of a specific genomic-proteomic signature that results from the completion of this organ's differentiation. R-hCG also inhibits the progression of early lesions, such as intraductal proliferations, and carcinomas in situ and the regression of established tumors. These observations indicate that r-hCG administered for a very short period of time has significant potential as a chemopreventive agent, protecting the normal cell from becoming malignant. This new biological concept also implies that when the genomic signature of protection or refractoriness to carcinogenesis is acquired, the hormonal treatment with r-hCG is no longer necessary. This is a novel approach that challenges the current concept that a chemopreventive agent needs to be given for a long period to suppress a metabolic pathway or abrogate the function of an organ.

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1. INTRODUCTION

SPORADIC BREAST CANCER is the fatal disease most frequently diagnosed in American women from all ethnic groups [1,2]. In 2002 the number of newly diagnosed invasive cancers was increased by approximately 6% from the previous year, reaching an approximate total of 203,500 cases [1,2]. Similar trends are observed worldwide, even in countries with low breast cancer incidence [3,4]. Although lower in incidence, a genetic predisposition to breast cancer represents a definitive threat, as women carrying germline mutations in the BRCA1 and BRCA2 genes are at an 85% lifetime risk of developing breast cancer, with a significantly earlier age of onset of the disease [5]. Although improved detection methods and diagnosis at an early stage have resulted in a decline in breast cancer mortality in the United States [1-3], all women with metastatic disease will die of breast cancer [6]. The incurability of the disease, in association with the worldwide increase in incidence indicates that primary prevention is the ultimate goal for breast cancer control [7-9].

The development of strategies for breast cancer prevention is hindered by the multistep nature of the process, and by the facts that only inheritance of cancer-predisposing genes [5,10-17] and radiation exposure at a young age [18-22] have been identified as mechanism or causal agents associated with cancer initiation. Current strategies to prevent breast cancer that focus on dietary changes to reduce fat intake to the levels of diets of countries with low breast cancer incidence are opposed by the argument that only a lifetime dietary change can decrease the risk of breast cancer. Therefore, major dietary changes undertaken now may not alter breast cancer incidence for another generation [23]. Another strategy capitalizes on a unique feature of breast cancer, its estrogen dependence, which can be manipulated to control growth or prevent tumor development utilizing either selective estrogen receptor modulators such as tamoxifen [10,12,24-26], or aromatase inhibitors such as arimidex, letrozole and exemestane [24,25]. However, the inability to predict who will develop breast cancer has required the implementation of broad, population-based strategies utilizing preventive measures that have significant side effects and require protracted treatment. These drawbacks have made these strategies not widely acceptable to a majority of treated women who would not have developed breast cancer even if untreated [27]. Therefore, what is needed is to precisely identify those women who should take a preventive agent, sparing others who will not develop the disease during their lifetimes. It is in this status of knowledge that we have developed a new paradigm for breast cancer prevention.

2. A NEW PARADIGM IN BREAST CANCER PREVENTION

Our paradigm has emerged from epidemiological observations of a direct association of breast cancer risk with nulliparity and of protection conferred by an early first full-term pregnancy [28-35]. We have chosen this specific strategy because it represents a window of opportunity that nature offers us for learning how a physiological event produces complete lifetime protection from breast cancer in a significant percentage of women. We are aware that although this physiological event does not explain all the questions about this complex disease, it provides a blue print for a new paradigm in breast cancer prevention. The novelty of this paradigm does not arise from the well-established knowledge that an early first full term pregnancy protects the breast against neoplastic transformation, but from our studies that have unraveled the biological principle underlying this protection [36-57]. We have demonstrated experimentally that the first pregnancy induces in the breast the expression of a specific genomic signature that results from the completion of a cycle of this organ's differentiation driven by the reproductive process. This signature, in turn, is a biomarker associated with a possible overall lifetime decreased breast cancer risk. More importantly, we have harnessed this biological principle by demonstrating in an experimental model that a short treatment with recombinant human chorionic gonadotropin (r-hCG), a placental hormone secreted during pregnancy, induces the same genomic signature that occurs in pregnancy, inhibiting not only the initiation but also the progression of mammary carcinomas, stopping the development of early lesions, such as intraductal proliferations, and carcinomas in situ (CIS). These

observations indicate that r-hCG administered for a very short period [36-50] has significant potential as a chemopreventive agent, protecting the normal cell from becoming malignant. This new biological concept also implies that when the genomic signature of protection or refractoriness to carcinogenesis is acquired, the hormonal treatment with hCG is not longer required. This is a novel concept that challenges the current knowledge that a chemopreventive agent needs to be given for a long period to suppress a metabolic pathway or abrogate the function of an organ [10,12].

3. DATA SUPPORTING THE NEW PARADIGM

The studies that support this paradigm and that are summarized below establish a baseline for understanding the evolution of glandular development, and how age and parity influence it. This knowledge is of utmost importance for understanding the role of differentiation in the protection of the mammary gland against carcinogenesis [51-53]. In addition, these data have identified biological endpoints for studying the response of the mammary gland to hormonal or chemopreventive agents, which could be utilized in modulating the susceptibility of the breast to carcinogenesis.

3.1. BREAST DEVELOPMENT AND THE PATHOGENESIS OF BREAST CANCER

The breast progressively develops from infancy to puberty under the main stimuli of pituitary and ovarian hormones. The least differentiated structure identified in the breast of postpubertal nulliparous women is the lobule type 1 (LOB 1), or TDLU, which progresses to lobule type 2 (LOB 2), which are composed of more numerous ductules per lobule and exhibit a more complex morphology [51-53]. During pregnancy, under the stimulus of new endocrine organs, the placenta and the developing fetus, the breast parenchyma branches profusely, leading to the formation of secretory lobular structures. During the 1st and 2nd trimesters of pregnancy LOB 1 and LOB 2 rapidly progress to lobule type 3 (LOB 3), which are composed of more numerous and smaller alveoli per lobule. During the last trimester of pregnancy active milk secretion supervenes, the alveoli become distended, and the lobules acquire the characteristics of the lobule type 4 (LOB 4), when the breast acquires a fully differentiated condition. LOB 4 is present throughout lactation. After weaning, all the secretory units of the breast regress, reverting to LOB 3 and LOB 2 [51-53]. After menopause all remaining differentiated lobular structures regress, acquiring the appearance of the LOB 1 of nulliparous women, from which it appears morphologically indistinguishable [51-54]. Nevertheless, the proliferative activity of LOB 1 of nulliparous women at menopause is 2-fold greater than that of the LOB 1 of parous women's breast [55].

3.2. PROLIFERATION, HORMONE RECEPTORS AND BREAST DIFFERENTIATION

The proliferative activity of the mammary epithelium varies as a function of the degree of lobular differentiation, which is in turn, driven by estrogens and progesterone, as well as by the hormones of pregnancy [54,56-61]. There is a progressive decrease in the percentage of proliferating cells that react positively to the Ki67 antibody (Ki67 or proliferation index) [53,54]. This decrease is associated with the progressive maturation of LOB 1 to LOB 2, LOB 3, and LOB 4, differences that are not abrogated when the proliferation index is corrected for the phase of the menstrual cycle [53,54]. Thus, parity, in addition to exerting an important influence in the lobular composition of the breast, profoundly influences the proliferative activity of the parenchyma. We have found that the proliferative activity and the percentage of cells positive for both ER and PR are highest in LOB 1, and both parameters progressively decrease in an inverse relationship to the degree of lobular differentiation, providing a mechanistic explanation for the higher susceptibility of these structures to be transformed by chemical carcinogens in vitro [62,63].

3.3. DIFFERENTIATION AND PATHOGENESIS OF BREAST CANCER

The breast of nulliparous women contains almost exclusively LOB 1, and their number remains nearly constant throughout the lifespan of the individual.

The breast of early parous women contains predominantly the more differentiated LOB 3, whereas LOB 1 are in a very low percentage until the fourth decade of life; thereafter their number starts to increase and after menopause they reach the same level observed in nulliparous women [52]. The fact that ductal breast cancer originates in LOB 1 [64,65] and the epidemiological observation that nulliparous women exhibit a higher incidence of breast cancer than parous women [32-35] indicate that LOB 1 in these two groups of women might be biologically different, or exhibit different susceptibility to carcinogenesis [51-54]. The confirmation in an experimental system that pregnancy and human chorionic gonadotropin (hCG) treatment confers long lasting protection, clearly indicates that the differentiation induced by these processes is a permanent modification of the biological characteristics of the mammary gland, in spite of the regression of differentiated structures to seemingly more primitive conditions [36-42,52,66].

3.4. DIFFERENCES IN THE GENOMIC SIGNATURE OF NULLIPAROUS AND PAROUS WOMEN'S BREAST

In order to determine whether the mammary epithelium in the normal breast of nulliparous women differs in its genomic profile from that of parous women, breast tissues free from pathological lesions were obtained from reduction mammoplasties performed in three nulliparous and three parous premenopausal women. Epithelial cells from LOB 1 and LOB 3 of nulliparous and parous women's breast, respectively, were microdissected by laser capture microdissection (LCM) [67]. RNA was extracted and hybridized to cDNA array membranes that contained 1,176 human genes (Clontech Human Cancer 1,2 Array).

The genomic signature of LOB 3 of parous women differed by 82 genes from that of LOB 1 of nulliparous women. Important differences were noted in the following genes: RhoE, PRL-1, IGFBP-3, and G/T mismatch-specific thymine DNA glycosylase gene. RhoE gene was increased by 14-fold in LOB 3 of the parous breast. This gene belongs to a small G-protein superfamily that consists of the Ras, Rho, Rab, Arf, Sar1, and Ran families [68-73]. In vivo, RhoE is found exclusively in the GTP-bound form, suggesting that unlike previously characterized small GTPases, RhoE may be normally maintained in an activated state [71]. This could be an important function, considering that; this gene might remain expressed even after involution of LOB 3 to LOB 1 in the postmenopausal state. PRL-1, or protein tyrosine phosphatase was significantly overexpressed (5-fold) in the LOB 3. This gene encodes a unique nuclear protein-tyrosine phosphatase [74] that is regulated by mechanisms different from those of other immediate-early genes such as c-fos and c-jun [75]. This gene has been shown to be upregulated in villus, but not crypt enterocytes, and in confluent differentiated but not in undifferentiated proliferating colon carcinoma cells [76], and in other systems it is also related to differentiation [77], development, and regeneration [78]. Therefore, its function in the breast epithelial cells of LOB 3 may be related to the differentiation process exhibited by these structures. Insulin-like growth factor binding protein-3 (IGFBP-3) was significantly overexpressed in LOB 3. This gene modulates the mitogenic and metabolic effects of IGFs, and forms a ternary complex with IGF-I or IGF-II and a 85-kD glycoprotein acid-labile subunit [79-81]. Interestingly, P53 may regulate apoptosis in tumor cells via transactivation of IGFBP-3 gene [82], indicating that in LOB 3 IGBP-3 may control differentiation by regulating programmed cell death. The G/T mismatch-specific thymine DNA glycosylase was 5-fold increase in expression in the LOB 3, data that led us to postulate that these more differentiated lobules had greater ability to repair DNA damage induced by any carcinogenic agent, whereas a lower reparative process in LOB 1 might be the determinant of higher susceptibility to be transformed. This postulate is supported by the higher frequency of ductal hyperplasia and carcinomas in situ arising in LOB 1. We have confirmed the differential expression of some of the genes that were overexpressed in LOB 3 using semiquantitative RT-PCR. We concluded that although the final biological significance of the genes found in the process of differentiation of the breast is not clear, the differences found can explain why the differentiated LOB 3 are resistant to grow in vitro and do not express transformation phenotypes upon carcinogen treatment, whereas cells from LOB 1 do [62,63].

3.5. ROLE OF HUMAN CHORIONIC GONADOTROPIN (hCG) IN BREAST CANCER PREVENTION

Our studies have revealed that the susceptibility of the mammary gland to be transformed by a chemical carcinogen is modulated by specific biological conditions of the host and of the target organ [83-85]. Tumor incidence and number of tumors per animal, which are the biological endpoints when evaluating tumorigenic response, are maximal when the carcinogen is administered to young but cycling virgin rats. Cancer incidence is directly proportional to the number of terminal end buds (TEBs) that are at their peak of cell proliferation [53,83-85]. Stimulation of the development and differentiation of the gland, resulting in profuse lobular development and depression of DNA synthesis [36-48,86], such as it occurs during pregnancy, or after completion of a 21 day-treatment of virgin rats with hCG, reduce the susceptibility of the mammary epithelium to be transformed by the carcinogen. The reduction in cancer incidence is permanent, as demonstrated by the similar degree of reduction when DMBA is administered after a delay of 21, 42, or 63 days after termination of hCG treatment.

3.6. EFFECT OF hCG ON MAMMARY CANCER PROGRESSION

Treatment with hCG after the carcinogenic process has been already initiated by DMBA inhibits the progression of mammary carcinomas. This phenomenon was evident by the reduction in number of preneoplastic lesions, such as ductal hyperplasia, and carcinoma in situ in hCG-treated animals [46-48].

3.7. COMPARISON OF THE EFFECT OF PREGNANCY AND hCG ON GENOMIC EXPRESSION AND SUSCEPTIBILITY OF THE MAMMARY GLAND TO CARCINOGENESIS

RNA was obtained from mammary glands of rats in their 15th and 21st day pregnancy or hCG treatment, and 21 and 42 days post-partum or treatment, respectively. RNAs were analyzed utilizing two membranes for each animal and compared with mRNA of age-matched virgin control rats. RNAs were hybridized to cDNA array membranes that contained 5,800 rat genes (Research Genetics, Alabama). Cluster analysis was performed using the Jaidexp (JAVA ANALYSIS INFORMATION & DATA EXPLORATION) specific program version 1:0 and statistically analyzed. Four clusters of genes were identified. CLUSTER A shows genes that were overexpressed (10-fold or more) at 15 and 21 days of pregnancy or hCG treatment, but decreased to control values after 21 and 42 days post-partum or treatment, respectively. These genes, which included β-casein and α-lactalbumin, are related to the secretory properties of the mammary epithelium [87]. CLUSTER B was composed of genes that were (3-fold or more) increased at 21 days of pregnancy/treatment and continued rising, reaching the highest peak at 21 days, decreasing by 42 days post-partum or hCG treatment. Among these genes were the fatty acid binding protein, the EST Rn.37635 with high homology to BCL7B gene, catechol-O-methyltransferase (COMT) gene, and the EST genes Rn.5953, Rn.22912 and Rn.4339 [87]. The upregulation of catechol-O-methyltransferase is significant because it can be involved in the conjugation of estradiol and estrogens, reducing the carcinogenic effect of these hormones. Genes related to the apoptotic pathways, such as testosterone repressed prostate message 2 (TRPM2), interleukin 1β-converting enzyme (ICE), bcl2, bcl-XL, bcl-XS, p53, p21, and c-myc were also up regulated from 3- to 5-fold [87]. We have shown that the activation of programmed cell death genes occurred through a p53-dependent process, modulated by c-myc and with partial dependence on the bcl2-family related genes [46-48]. In this cluster were also included inhibins A and B, heterodimeric non-steroidal secreted glycoproteins with tumor suppressor activity [88,89]. We have found that inhibins are not present in the normal resting mammary gland, but are induced by pregnancy or hCG treatment [45,88]. We have also shown that hCG has an autocrine or paracrine effect on mammary epithelial cells [46]. hCG also activates the CLUSTER B of genes in DMBA-induced mammary tumors, indicating that this hormone acts through the same pathways for exerting its preventative and therapeutic effects. CLUSTER C represents genes whose level of expression progressively increased with time of pregnancy or

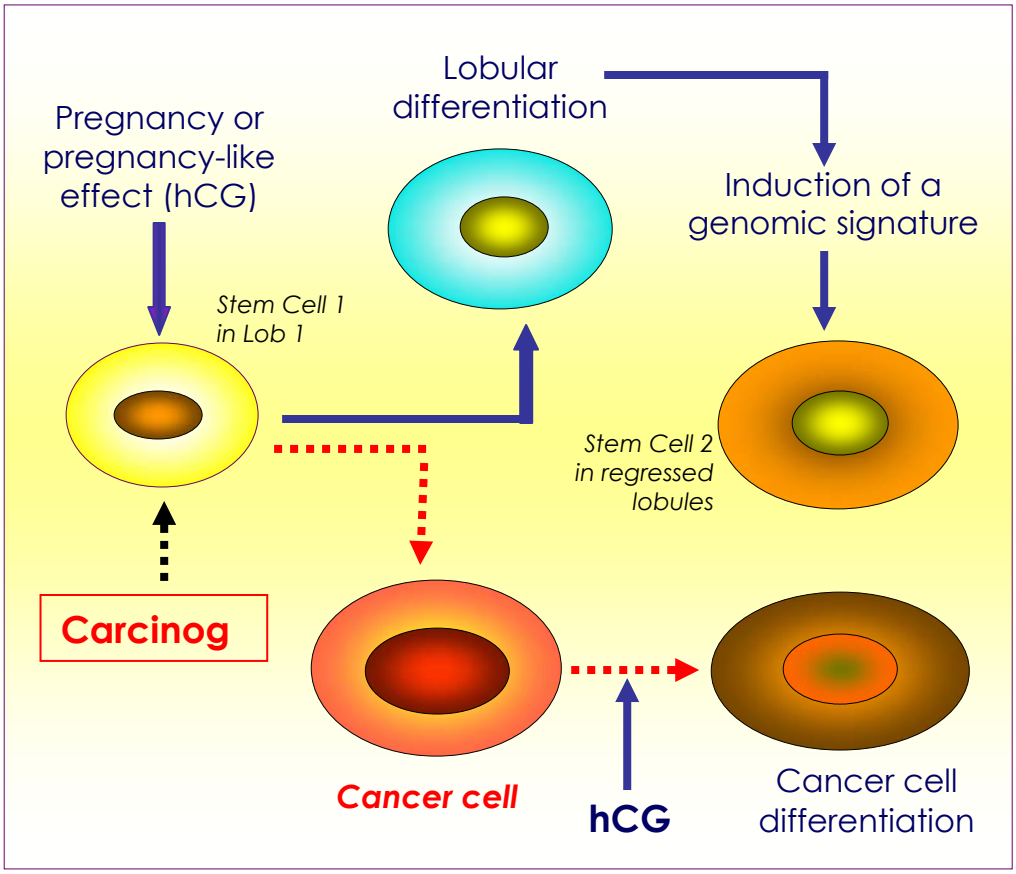

FIGURE 1. DIFFERENTIATION INDUCES A SHIFT OF THE STEM CELL 1 TO A STEM CELL 2 THAT IS REFRACTORY TO CARCINOGENESIS.

hCG treatment, reaching their highest levels between 21 and 42 days post-partum or end of treatment. Among these were known genes such as those coding for a fragment of glycogen phosphorylase, AMP activated kinase, bone morphogenetic protein 4 and vesicle-associated protein 1 [87]. G/T mismatch-specific thymine DNA glycosylase gene, which was observed to be upregulated in the LOB 3 of the human breast, was also increased by 5-fold in this model. These data indicate that the activation of genes involved in the DNA repair process is part of the signature induced in the mammary gland by either pregnancy or hCG treatment of virgin animals. These observations confirm our previous findings that in vivo the ability of the cells to repair carcinogen-induced damage by unscheduled DNA synthesis and adduct removal is more efficient in the parous and in the hCG-treated virgin than in the untreated virgin animal mammary gland [84,90-92]. Therefore, a principal mechanism mediating the protection from mammary carcinogenesis conferred by either full term pregnancy or hCG treatment is the enhancement of the ability of the cells to repair DNA damage, which is in turn the determinant of the lower susceptibility to carcinogenesis. CLUSTER D consists of genes coding for pro-alpha collagen III, procollagen II-α1, BTG1 protein and thymosin-β4, which were upregulated more than (3-fold at the 15th day of pregnancy or hCG treatment, downregulated at the 21st day in both pregnant and hCG-treated animals, and remained downregulated up to 42nd days [87]. CLUSTER D, in combination with CLUSTER C, is a component of the signature induced by hCG in the mammary gland (FIG. 1).

These data demonstrate that the genomic signature of the mammary gland induced in virgin animals by exogenous administration of hCG is

similar to that induced by pregnancy, and that specific genomic profiles are still manifested by 42 days post termination of treatment [87]. The importance of these specific signatures is highlighted by the fact that administration of carcinogen to hCG-treated or control virgin rats whose mammary glands appear morphologically similar will induce a markedly different tumorigenic response, supporting the concept that the differentiation induced by hCG is expressed at genomic level, and results in a shift of the susceptible STEM CELL 1 to a refractory STEM CELL 2 (see FIG. 1). The permanence of these changes, in turn, makes them ideal surrogate markers for the evaluation of hCG effect as a breast cancer preventive agent.

3.8. EFFECT OF RECOMBINANT hCG ON PRIMARY BREAST CANCER

Based on our preclinical data that had demonstrated that r-hCG treatment of virgin rats prevented the initiation and inhibited the progression of DMBA-induced mammary carcinomas [36-42,47-49], we designed a pilot study for evaluating the effect of r-hCG on primary breast cancer in post-menopausal patients [50]. In a double-blind, placebo-controlled study, 25 post-menopausal women with primary operable breast cancer (T1-T3) whose diagnosis was made by core biopsy performed on day 0, received on alternate days for 2 weeks intramuscular injections of either r-hCG (500 g, N=20) or placebo (N=5). Surgery (mastectomy or lumpectomy) was performed on day 15. The tumor tissue obtained in the initial core biopsy and that removed at the time of therapeutic surgery were evaluated to determine the rate of cell proliferation, or proliferative (Ki67) index and inhibin immunoreactivity.

The most remarkable effects attributed to this two-week treatment were a significant reduction in Ki67 index from 18% in the initial biopsy to 4% in the mastectomy/ lumpectomy specimens ($P<0.00006$), and increased synthesis of inhibin. Serum hormonal levels were those characteristics of post-menopausal women, and remained unchanged during and after the treatment, except for elevation in hCG levels during treatment. Serum levels of progesterone, 17β-estradiol, SH and LH were not affected by the hormonal treatment. Hormone administration was well tolerated by all patients, and no local or systemic side effects were reported at any time.

4. UNIFYING CONCEPTS

Breast cancer originates in the undifferentiated terminal duct of the LOB 1 that contains stem cells (STEM CELL 1 in FIG. 1) the site of origin of ductal carcinomas. The susceptibility of LOB 1 to undergo neoplastic transformation has been attributed to its high rates of cell proliferation and of carcinogen binding to the DNA and low reparative activity [66,90-94]. The hormonal milieu of an early full term pregnancy or hCG treatment induces lobular development, completing the cycle of differentiation of the breast. This process induces a specific genomic signature in the mammary gland that is represented by the STEM CELL 2 (FIG. 1). 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. The STEM CELL 2 is able to metabolize the carcinogen and repair the induced DNA damage more efficiently than the STEM CELL 1, as it has been demonstrated in the rodent experimental system [84,90-92]. We also have evidence that hCG has an effect in the cancer cell by further the differentiation pattern [50] (FIG. 1). The finding that differentiation is a powerful inhibitor of cancer initiation provides a strong rationale for identifying the genes that control this process. The basic biological concept is that pregnancy or hCG shifts the STEM CELL 1 to the STEM CELL 2 that is refractory to carcinogenesis.

This concept is supported by the laboratory findings described in the previous sections and summarized below:

1) Based in our knowledge of the pathogenesis of mammary cancer we have tested the effect of hCG hormone on the early phases of tumor progression, namely from TEBs damaged by DMBA to IDPs, in situ carcinomas and invasive carcinomas and demonstrate that this hormone inhibits the progression of DMBA-induced mammary tumors.

2) Treatment of young virgin rats with hCG

induced a profuse lobular development of the mammary gland, practically eliminating the highly proliferating TEBs, with an overall reduction in the proliferative activity of the mammary epithelium, and induction of the synthesis of inhibin, a protein with tumor-suppressor activity.

3) The hormonal treatment induced differentiation of the mammary gland, which was manifested at morphological, cell kinetic and functional levels. The morphological changes consisted of progressive branching of the mammary parenchyma and lobule formation. They were accompanied by reduction in the rate of cell proliferation.

4) The functional changes comprised increased synthesis of inhibin, β-casein and other milk-related bioactive peptides. In addition, hCG also increased the expression of the programmed cell death TRPM2, ICE, p53, c-myc, and bcl-XS, inducing as well apoptosis, and down regulation of cyclins. Programmed cell death genes were activated through a p53-dependent process, modulated by c-myc, and with partial dependence on the bcl-2 family-related genes.

5) hCG action treatment activates known and new genes. For this purpose we have used a differential display technique that allowed us to identify a series of new genes, among them the gene 19, 29 and 44 in the hCG-treated MCF-7 cells and the hormone-induced-1 (HI-1) in the rat mammary gland of animals treated with hCG. HI-1 might be of potential for clarifying the mechanisms through which hCG inhibits the initiation and progression of mammary cancer. Of relevance was the observation that lobular development, which reached its maximal expression after the 15^{th} day of hCG treatment, regressing after hormone withdrawal, was preceded by activation of genes associated with the expression of programmed cell death, and furthermore, that the expression of these genes, including the newly identified gene HI-1, was still elevated 20 days post-cessation of treatment.

6) Data generated with the new tools provided by the cDNA microarray techniques have allowed to demonstrate that while lobular development regressed after the cessation of hormone administration, programmed cell death genes remained activated, but more importantly a new set of genes (CLUSTER D) reached the maximum expression whereas other (CLUSTER C) are down-regulated. Those genes in CLUSTER C and D are the one that are providing the genomic signature that is specific for hCG and pregnancy.

7) The genomic signature is specific for pregnancy and hCG and significantly different than the one induced by y other hormones such as estrogen and progesterone.

These mechanisms play a role in the protection exerted by hCG from chemically induced carcinogenesis, and might be even involved in the life-time reduction in breast cancer risk induced in women by full term and multiple pregnancies. The implications of these observations are 2-fold: on one hand, they indicate that hCG, as pregnancy, may induce early genomic changes that control the progression of the differentiation pathway, and that these changes are permanently imprinted in the genome, regulating the long-lasting refractoriness to carcinogenesis. The permanence of these changes, in turn, makes them ideal surrogate markers of hCG effect in the evaluation of this hormone as a breast cancer preventive agent.

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ABBREVIATIONS USED:

hCG and r-hCG, human chorionic gonadotropin and the recombinant hCG, respectively; CIS, carcinomas in situ; LOB, lobule type; TDLU, terminal ductal lobular unit; TEB, terminal end buds; DMBA, 7,12-dimethylbenz(a)anthracene; EST, expressed sequence tag(s); LCM, laser capture microdissection; IGFBP-3, insulin-like growth factor binding protein-3; TRPM2, testosterone repressed prostate message 2; ICE, interleukin 1β-converting enzyme; HI-1, hormone-induced-1; SH, somatotropin; LH, luteinizing hormone; ER, estrogen receptor; PR, progesterone receptor.

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