Jose Russo · Irma H. Russo

Role of the Transcriptome in Breast Cancer Prevention

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We dedicate this book to all my trainees from whom we have received more than they ever have expected to give us. Among them Jwang Ling M.D., Eugene Agnone M.D., Francisco Martinez M.D., Gloria Calaf Ph.D., Daniel R. Ciocca M.D., Lee K. Tay Ph.D., Stephen P. Ethier Ph.D., Gustavo A. Moviglia M.D., Satori Higa M.D., Megan Mills Ph.D., Gustavo Rubio Coronel M.D., Anna Sapino M.D., Fulvio Basolo M.D., Gabriella Fontanini M.D., Josiah Ochieng Ph.D., Muneesh Tewari M.D., Ph.D., Pei-Li Zhang Ph.D., Maria Elena Alvarado M.D., Anthony Magliocco M.D., Teh-Yuan Ho Ph.D., Nandita Sohi Ph.D., Chai Yu-Li M.D., Saad El-Gendy Ph.D., Judith Gordon M.D., Eric G. Thomas D.O., Roupen Yagsessian M.D., Kunle Adesina M.D., Ph.D., Ruth Padmore M.D., Ph.D., Yun-Fu Hu Ph.D., Ana Maria Salicione Ph.D., Betsy Bove Ph.D., Yajue Huang Ph.D., Ismael Dale Cotrin Silva M.D., Ph.D., Abdel-Rahman N. Zekri M.Sc., Ph.D., Xiaoshang Jiang Ph.D., Raquel Angela Silva Soares Lino Ph.D., Hasan M. Lareef M.D., Gabriela Balogh Ph.D., Fathima Sheriff M.D., Sandra Fernandez Ph.D., Johana Vanegas M.D., Raquel Moral Ph.D., Daniel Mailo Ph.D., Ricardo Lopez Ph.D., Julia Pereira Ph.D., Hilal I. Kocdor M.D., Ph.D., Mehmet A. Kocdor M.D., Sylvana Carrea de Noronha Ph.D., Samuel Ribeiro de Noronha Ph.Dc., and Yanrong Su Ph.D.

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Contents

Chapter 1
The Epidemiology of Breast Cancer and the Basis for Prevention

1.1 Introduction

Breast cancer is a heterogeneous and complex disease resulting from the uncontrolled growth of cells that are unique and specific to the breast. The occurrence of cancer of the breast has long been known, as documented in the Edwin Smith surgical papyrus, written between 3000 and 1500 BC [1] (Table 1.1). Although nowadays breast cancer is the malignant disease most frequently diagnosed in postmenopausal Caucasian women born in Northern European countries and in America [2–4], the disease affects women of all races and nationalities. Furthermore, its incidence around the globe is increasing in countries that are industrialized [3], as well as in those that have recently become industrialized [5, 6]. The worldwide incidence of breast cancer has increased 30–40% since the 1970s, reaching a total of 1,383,500 new cases and a mortality of 458,400 cases by 2011 [2, 4, 7].

Although the definitive cause or mechanisms behind initiation of breast cancer, whose knowledge is essential for developing strategies for its prevention, have not been identified, epidemiological, clinical, and pathological studies have uncovered novel aspects regarding the complexity of this disease [8–10]. Among these, the knowledge that age at diagnosis and ethnicity are associated with a specific tumor type and tumor behavior, and that they are in turn differently influenced by a woman's age at the first pregnancy [11, 12], indicate that the global incidence of breast cancer changes over time in relation to geography, race, and changes in lifestyle, suggesting that breast cancer risk is influenced by a multiplicity of still undefined factors.

The task at hand is to identify amongst a multiplicity of still undefined factors which ones are etiologically relevant in breast cancer risk. A common denominator for the risk of developing breast cancer has been found to be a reproductive history [8, 9, 12]. Increased breast cancer incidence and mortality were associated with nulliparity as early as the 1700s, as reported by Bernardino Ramazzini, who attributed the phenomenon to the childlessness of nuns in Italian convents [13]. MacMahon et al. [8] reported that pregnancy exerted a protective effect in women whose first

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Table 1.1 Landmarks in the history of breast cancer

Year	Landmark discovery or observation
3000–1500 BC	Edwin Smith surgical papyrus describes eight cases of breast cancer in women
1700	Bernardino Ramazzini attributed the high incidence of breast cancer in nuns to their childlessness
1896	Beatson demonstrated that removal of the ovaries caused regression of advanced cancer in young women
1959	Huggins et al. demonstrated in laboratory animals that chemical carcinogens induced mammary cancer in rats, and that removal of the pituitary or the ovaries reduced tumor size
1970	MacMahon et al. reported that pregnancy exerted a protective effect in women whose first child was bore from early teens to the middle 20s
1975	Soule develops the breast cancer cell line MCF-7, which was instrumental for the discovery of the ERα
1978	Russo and Russo establish the concept the mammary gland differentiation induce by full-term pregnancy is the mechanism behind the protective effect against breast cancer

child was born from the early teen years to the middle 20s relative to a risk of 100 for nulliparous women (Table 1.1). Numerous studies have confirmed these results and have additionally reported that multiple pregnancies significantly decrease the risk of developing breast cancer after 50 years of age [8, 9] (Fig. 1.1), whereas postponement of the delivery increases a woman's breast cancer risk, which reaches the same levels observed in nulliparous women when it occurs between 30 and 34 years of age, increasing even further after 35 years [8, 9] (Fig. 1.2). An understanding of the mechanisms that determine whether a pregnancy would prevent breast cancer or would increase its risk requires taking into consideration not only the age at the first pregnancy but also the age at the time of breast cancer diagnosis, which in turn influences the stage and pathological characteristics of the tumors developed [14–16] (Table 1.1).

Pregnancy itself is a complex process that only succeeds when a woman's ovaries are fully functional and secrete estrogen and progesterone, hormones that are essential for the maintenance of pregnancy. The ovaries work under the control of the hypothalamic–pituitary–gonadal (HPG) axis [17, 18], which synchronizes the ovarian secretions with those of pituitary and placental hormones for stimulating breast development in preparation for milk production [18, 19]. Primiparous women younger than 25 years old that have elevated serum levels of hCG during the first trimester of pregnancy have a 33% decrease in risk of breast cancer diagnosis after the age of 50, whereas estrogen concentrations have been positively associated with risk of breast cancer before age 40, supporting the role of this or other pregnancy hormones in the development of breast cancer [12, 20–24] (Table 1.2).

An understanding of the relationship between pregnancy and environmental influences on breast cancer risk requires the use of experimental models that have already contributed to unraveling some of the endocrinological mechanisms mediating cancer prevention when the reproductive event precedes carcinogen exposure

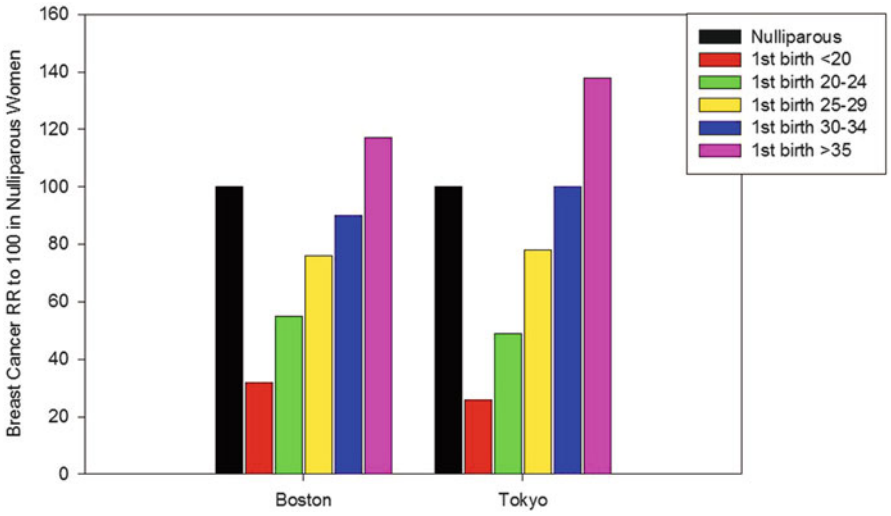

Fig. 1.1 Breast cancer relative risk (RR) by age at first pregnancy. The histogram depicts breast cancer incidence in two out seven different regions of the world (adapted from [8])

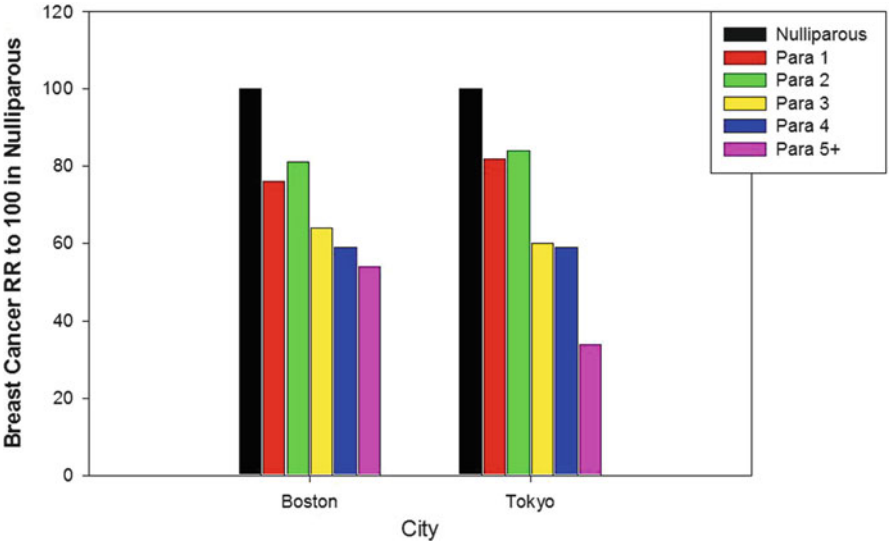

Fig. 1.2 Breast cancer relative risk (RR) by number of pregnancies. The histogram depicts breast cancer incidence in two out seven different regions of the world (adapted from [8])

[22, 25–28], or acts as a promoter of mammary cancer when it is initiated after carcinogen-induced damage has been initiated [29–31]. These models have also contributed to uncover the preventive effects of estrogen on chemically induced carcinogenesis when given alone at a moderate dose to prepubertal rats [32], or as

Table 1.2 Who is at risk in breast cancer today—a modern epidemic

Clinical relevance	Supporting data
Breast cancer as an age-related disease	Sporadic breast cancer: 95% of new cases are sporadic Diagnosed after age 50 in postmenopausal women with no family history of the disease Tend to be of low grade and predominantly estrogen receptor (ER) positive ductal carcinomas Are responsive to antiestrogen and immunotherapy
Breast cancer diagnosis before menopause	Represents ~5% of all cases Diagnosed predominantly in women with positive family history or proven inheritance of deleterious mutations in the TP53, AT, BRCA1 or BRCA2 genes. Inheritance of BRCA1/2 mutations are more frequently in women of Ashkenazi Jew or African ancestries, and in association with cancer diagnosis before age 40 The cancers developed are basal-like triple negative, characterized by absence of ER, progesterone receptor (PR), and Her2, and therefore unresponsive to endocrine or immunotherapy
Pregnancy associated breast cancer (PABC)	Diagnosed during pregnancy or within 1 or 2 years following delivery. PABC has a worse prognosis and more pronounced mortality than breast cancer diagnosed to women with no PABC The hormonal milieu of pregnancy might stimulate the progression of preexisting preneoplastic lesions
Protective effect of first full-term pregnancy from developing breast cancer after menopause	Having first child from early teens to middle 20s (early parity) Breastfeeding Multiparity in early parous women Each additional birth confers greater protection
Human chorionic gonadotropin (hCG)	Women first pregnant before age 25 with high levels of hCG during the first trimester of pregnancy have a 33% decrease in risk of breast cancer diagnosis after the age of 50

therapeutic agents when given in combination with progesterone to carcinogen-exposed rats [33]. Various natural and synthetic hormones have been reported to inhibit the progression of chemically induced mammary cancer in different strains of virgin rats [34] and in mouse models of mammary carcinogenesis [35–37]. The development of genetically engineered mice (GEM) models has greatly contributed to the understanding of gene–environmental interactions, finding that has been reported extensively in excellent publications [38–43], therefore this field will be only summarily addressed in this chapter.

The identification of the specific conditions under which the completion of one pregnancy at early age fully differentiates the breast epithelium and reduces breast

cancer risk should serve as a blueprint for understanding the anatomical, physiological, endocrinological, and molecular mechanisms that need to be operational for developing rational strategies for the prevention of breast cancer in future generations. In the present chapter we will first address the strategies utilized in the rodent experimental system, secondly, we will review our knowledge on pregnancy and cancer in women, and lastly, we will address all of the other confounding factors that we need to bear in mind for understanding and solving the complex and fascinating relationships between pregnancy and cancer.

1.2 Rodent Models of Mammary Carcinogenesis

Spontaneous mammary tumors are frequently observed in long-term rodent studies [30, 44]; however, their usefulness for carcinogenicity testing is hindered by the tumors' biological characteristics, such as long latency period, variations in etiologic agents or mechanisms of tumor initiation, and pregnancy dependence in certain strains of mice [45]. The induction of hormone-dependent rat mammary tumors with chemical carcinogens, on the other hand, has become an essential model for testing the carcinogenic potential of specific chemicals, such as 3,4-benzopyrene, 3-methylcholanthrene (MCA) [46] and the polycyclic aromatic hydrocarbon (PAH) 7,12-dimethylbenz(a)anthracene (DMBA) [47], or the alkylating agent *N*-methyl-*N*-nitrosourea (MNU) [48, 49]. Chemically induced tumors developed in mice strains of low spontaneous mammary cancer incidence or in transgenic mice are adenoacanthomas or type B adenocarcinomas that are in general estrogen receptor alpha (ERα) negative [43]. However, in p53 null mice hormonal stimulation by estrogen and/or progesterone or prolactin/progesterone, markedly enhances tumorigenesis, whereas blocking estrogen signaling through ovariectomy or tamoxifen treatment greatly reduces the tumorigenic capability of the mammary epithelium, an indication that normal mammary gland and preneoplastic lesions are responsive to estrogen [43]. The majority of rat mammary tumors induced by DMBA or MNU are ductal adenocarcinomas that are ERα positive and reproduce the pathological features of the most frequent type of adenocarcinomas developed by women [50]. The characteristics of this model have opened a myriad of opportunities for dissecting the initiation, promotion, and progression steps of carcinogenesis and for translating these findings to the human situation [22, 30, 50].

1.3 Windows of Susceptibility to Carcinogenesis

The response of the mammary gland to specific carcinogenic stimuli depends upon the physiologic state of the mammary tree under the control of the endocrine system. The administration of optimal carcinogenic doses to young and sexually mature

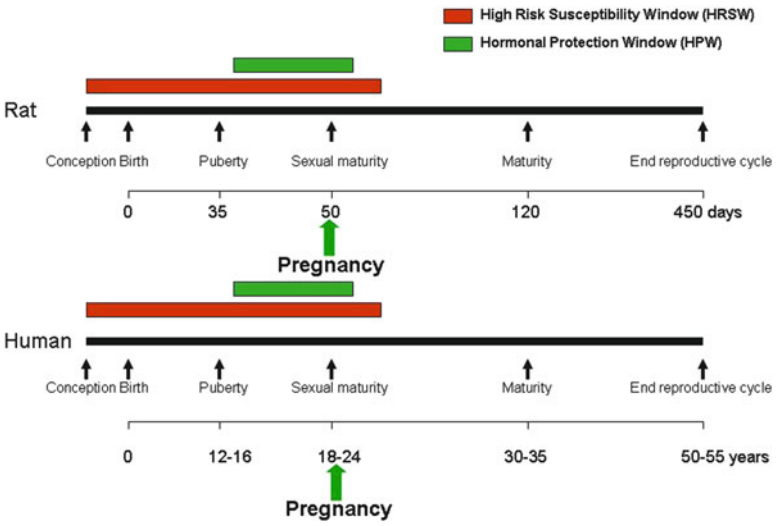

Fig. 1.3 Diagrammatic representation of mammary gland development from conception to the end of reproductive life. In both rats (*upper line*) and humans (*lower line*) the period of life that begins in uterus and persists until sexual maturity, represents a window of greater susceptibility of the mammary gland to be damaged by exogenous carcinogenic stimuli or exposure to endocrine disruptors. The differentiation of the mammary gland induced by pregnancy or the appropriate hormonal treatments needs to occur during the post-pubertal period and before the mammary epithelium has suffered any damage, representing a hormone-driven window of protection that overrides the high risk window. *HRSW* high risk susceptibility window, *red bar*; *HPW* hormonal protection window, *green bar* (from Russo IH, Russo J (2011) Pregnancy-induced changes in breast cancer risk. A review. J Mammary Gland Biol Neoplasia 16:221–233)

virgin rats induces maximal tumorigenic response [29, 30, 46–49]. This period of highest susceptibility of the mammary gland to be transformed by such stimulus represents the "high risk susceptibility window" (HRSW), which encompasses different stages of development, i.e., prenatal life, infancy, puberty, and early adulthood (Fig. 1.3). Thus, in addition to age, the tumorigenic response elicited by carcinogenic agents is modulated by the animal's endocrinological milieu prevailing at the time of exposure, as well as by endocrine and environmental influences occurring during the HRSW [51–54]. The peak of cancer incidence occurring when virgin rats reach the age of 45–55 days and have had at least two ovulatory cycles after vaginal opening [55], represents the response of numerous mammary terminal end buds (TEBs) that are predominantly composed of progenitor mammary stem cells (PMSCs). These cells have been characterized by their size, nuclear-cytoplasmic ratio and euchromatin-heterochromatin ratio, number and distribution of organelles, and proliferative activity [30, 56]. Under normal conditions, PMSCs cells are primed by ovarian hormones for expansion of the mammary parenchyma and lobular formation. Instead, when they are exposed to a carcinogen such as DMBA, the PMSCs exhibit the highest rate of carcinogen uptake and of cell proliferation [30]. Within a few days transformed PMSCs expand and form intraductal proliferations (IDPs) that

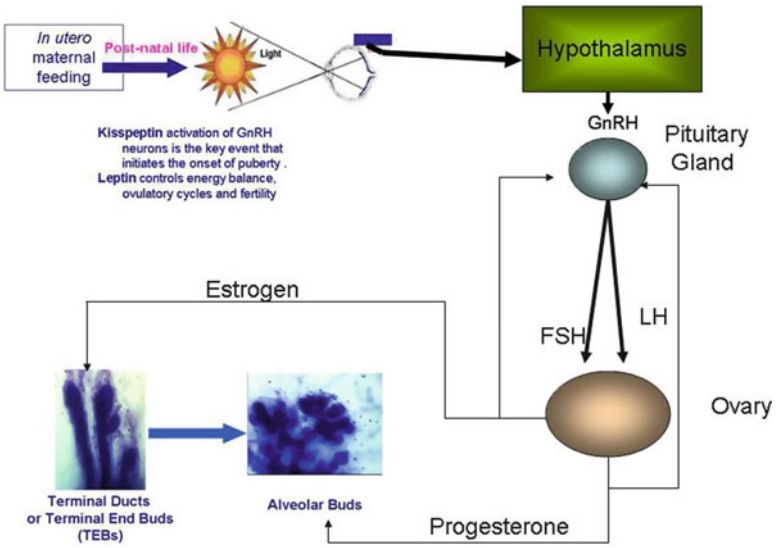

Fig. 1.4 Hypothalamic effect on gland development

progress to ductal carcinomas in situ and invasive, confirming the transition of PMSCs to mammary cancer stem cells (MCSC) under the influence of a carcinogen [56, 57]. Morphologically similar cells have been isolated from DMBA-induced mammary tumors [58]. In the mouse, the mammary gland continually undergoes postnatal developmental changes that are driven by signals from TEBs [59]. They direct ductal growth and elongation, producing a progeny of varied lineages that include luminal and myoepithelial cells under the influence of signals from the local tissue microenvironment [59].

1.4 Prevention of Mammary Cancer by Pregnancy

Under the stimulus of the first pregnancy, the mammary gland that has not been exposed to a carcinogenic insult during the early phases of the HRSW enters into a "hormonal protection window" (HPW). During this period the hormones of pregnancy will block any future damage caused by carcinogens or endocrine disruptors through the induction of mammary gland differentiation [22, 30] (Fig. 1.3). The first pregnancy is an essential step for determining the fate of the mammary gland future cancer risk. Its success depends on timely ovulation, which in turn, is the result of a sequence of neuroendocrine events (Fig. 1.4) triggered within the preoptic area (POA) and the mediobasal hypothalamus by the positive feedback of estrogen

secreted by ovarian follicles and metastin (KISS-1), a natural ligand for the G protein-coupled receptor GPR54 [60, 61]. This stimulus induces the surge of gonadotropin releasing hormone (GnRH) and of luteinizing hormone (LH) for triggering ovulation. After oocyte fertilization and implantation, ovarian estrogen, progesterone, and inhibin are supplemented by rat chorionic gonadotropin and rat placental lactogen, hormones synthesized by the developing embryo and the placenta. Jointly they contribute to stimulate the mammary glands to undergo active cell proliferation and differentiation of TEBs to alveolar buds (ABs) and lobules. The contributions of the pituitary hormones prolactin (PRL) that stimulates milk production and oxytocin that enhances the secretory activity of the alveolar cells complete the functional differentiation of the mammary gland [18, 62]. Completion of pregnancy and lactation induce long-lasting structural and genomic changes in the mammary gland of different strains of rats and in mice [33]. These molecular changes ultimately result in a significant reduction in mammary cancer incidence and number of tumors per animal [25–28, 55, 56].

1.5 Prevention of Mammary Cancer by Hormones

In the absence of pregnancy, various natural and synthetic hormones have been shown to prevent the initiation of mammary cancer when they are administered to young virgin rats during the HRSW prior to the exposure to a carcinogen or an endocrine disruptor. Prepubertal administration of 10 μg 17α-estradiol to Sprague–Dawley rats significantly advances the age at vaginal opening, stimulates lobular development of the mammary gland, and reduces the incidence of DMBA-induced tumors [32]. Norethynodrel-Mestranol (NM) administered at a contraceptive and a tenfold higher dose to post-pubertal and sexually mature virgin rats result in long-lasting structural changes in the mammary gland and a dose-dependent reduction in tumor incidence [63]. Daily treatment of virgin Sprague–Dawley rats with hCG at the doses of 1, 5, 10, or 100 IU for 21 days significantly reduces adenocarcinoma incidence and number of adenocarcinomas per animal in a dose-dependent manner [22] (Fig. 1.5). Similarly, treatment with 100 IU hCG daily for 5, 10, or 15 days suffices to induce a significant degree of mammary gland differentiation and protection from cancer initiation [64] (Fig. 1.6). The reduction in cancer incidence resulting from hCG treatment is long-lasting, as demonstrated by the persistent reduction in carcinogenic response to administration of DMBA at 21, 42, or 63 days after termination of the hormonal treatment [22, 26]. In spite of the morphological regression of the mammary gland after cessation of the hormonal treatment, the analysis of mRNA reveals elevated expression of genes involved in the apoptotic pathways, which include testosterone repressed prostate message 2 (TRPM2), interleukin 1β-converting enzyme (ICE), bcl-XL, bcl-XS, p53, p21, and c-myc [65], as well as activation of tumor suppressor activity through upregulation of inhibin A and B [66, 67].

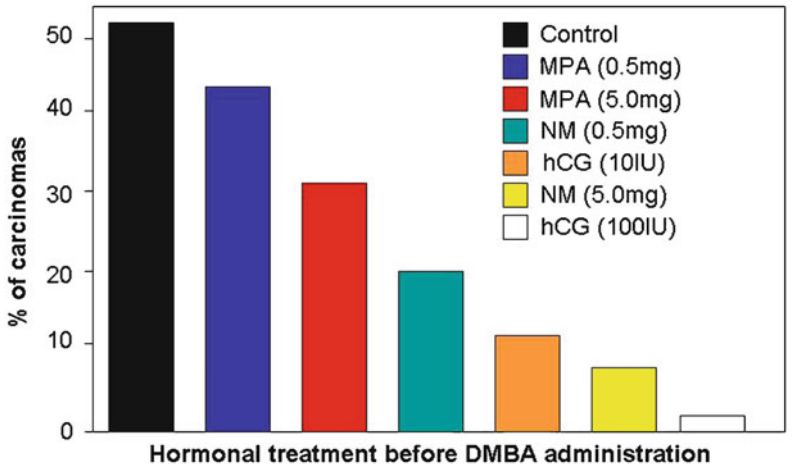

Fig. 1.5 Effect of hormonal treatment before induction of mammary cancer with dimethylbenz(a) anthracene (DMBA)

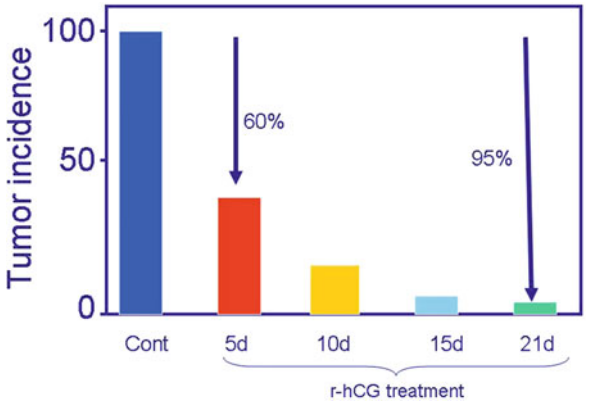

Fig. 1.6 Treatment of virgin rats with recombinant hCG prevents DMBA-induced mammary carcinogenesis

1.6 Effect of Pregnancy on Cancer Progression

It is important to take into consideration the fact that the protective effect of pregnancy or hormones acting during the HPW might be nullified if the mammary gland has been exposed to environmental carcinogens or endocrine disrupting agents before or during the early phases of the HRSW (Fig. 1.3). Pregnancy initiated 15 days after DMBA or MCA feeding [29, 68, 69] induces 100% incidence of

mammary carcinomas; the tumors rapidly grow during the gestational period and are maintained by lactation. The nursing stimulus maintains the growth of DMBA-induced rat mammary carcinomas, whereas cessation of nursing causes tumor regression [68]. The enhanced growth of mammary carcinomas by pregnancy has been attributed to increased secretion of estrogen, progesterone, prolactin, placental lactogen, or relaxin [29]. Although all of these hormonal influences might be of importance in promoting the growth of mammary carcinomas, results are inconclusive, and sometimes controversial. Grubbs et al. [70] found decreased cancer incidence in MNU-treated rats after pregnancy or pregnancy and lactation, although the time of appearance of the first palpable cancers was shorter in rats becoming pregnant 10 days after carcinogen administration [70].

1.7 Effect of Hormones on Tumor Progression

The hormone dependence of breast cancer that had been established by Beatson [20] (Table 1.1) was not recognized in laboratory animals until Huggins et al. [46] demonstrated that all 3-MC treated rats exhibited a deep reduction of tumor size after hypophysectomy. Ovariectomy also reduced mammary cancer incidence by 40%; administration of daily injections of 0.1 or 0.2 μg 17β-estradiol increased mammary cancer incidence to 100%, whereas rats receiving 20 μg 17β-estradiol daily had a 70% reduction in incidence. Dihydrotestosterone treatment also decreased tumor size; whereas progesterone or diethylstilbestrol administered to ovariectomized rats increased tumor incidence and enhanced the speed of tumor growth. Blocking the action of estrogens by antiestrogens that bind to the ERα, such as tamoxifen [71] has demonstrated a long-lasting chemopreventive effect on mammary tumors both benign and malignant. Nevertheless, hormone-independent tumors continue growing after ovariectomy as well after prolonged treatment with tamoxifen [46, 71] (Table 1.3).

Numerous treatments have been developed for the extinction of chemically induced tumor in rodents. DMBA-treated Sprague–Dawley rats that begin receiving a daily injection of 100 IU hCG 20 days after carcinogen administration exhibit a significant reduction in mammary adenocarcinoma incidence and number of tumors per animals, an effect that becomes evident as early as 10 days after initiation of the hormonal treatment and persisted for 40 days after its termination [22, 25, 26] (Table 1.3). Treatment of various strains of rats with hormonal combinations, i.e., ethinyl estradiol-megestrol acetate; ethinyl estradiol-norethindrone [29, 30] or 17β-estradiol-progesterone [33] 2 weeks after NMU administration significantly inhibits tumor progression. Protection conferred by 17β-estradiol and progesterone to BALB/c mice after treatment with DMBA administration is associated with activation of p53 in response to the hormonal treatment, which is sustained to induce p21 upon carcinogen challenge [36, 40] (Table 1.3).

Table 1.3 Modulators of carcinogenic response

Biological or clinical event	Supporting data
Pregnancy after carcinogen exposure	Pregnancy initiated 15 days after DMBA or MCA feeding induces 100% incidence of mammary carcinomas [29]
	The tumors rapidly grow during the gestational period [68]
	The nursing stimulus maintains the growth of carcinogen-induced rat mammary carcinomas [69]
	Cessation of nursing causes tumor regression
Hormonal treatments after carcinogen exposure	Dihydrotestosterone—Decreases tumor size
	Progesterone or diethylstilbestrol—Administered to OVEX rats increase tumor incidence and enhance the speed of tumor growth
	Antiestrogens (Tamoxifen)—Exert a long-lasting chemopreventive effect on mammary tumors
	Ovariectomy (OVEX)—3-MC treated rats exhibit reduced mammary cancer incidence by 40% after OVEX
	17β-estradiol—Daily injections of 0.1 or 0.2 μg 17β-estradiol increase mammary cancer incidence to 100%. Daily injections of 20 μg 17β-estradiol decrease mammary cancer incidence by 70%
	Hormone-independent tumors continue growing after OVEX as well after prolonged treatment with tamoxifen
	Human chorionic gonadotropin (hCG)—Virgin Sprague–Dawley rats that receive a daily injection of 100 IU hCG starting 20 days after DMBA administration exhibit complete extinction of carcinomas in situ and significant reduction in incidence of invasive adenocarcinomas. The effect becomes evident as early as 10 days after the initiation of hormonal treatment and persists for 40 days after its termination
	17β-Estradiol-progesterone combination—Treatment of strains of rats that differ in susceptibility to carcinogens (Lewis, Wistar-Furth, Fischer 344, and Copenhagen) starting 2 weeks after NMU administration significantly inhibits tumor progression in all the strains
	Ethynyl estradiol-megesterol acetate and ethynyl estradiol-norethindrone—Administered 2 weeks after NMU treatment significantly inhibit tumor progression

(continued)

Table 1.3 (continued)

Biological or clinical event	Supporting data
Age at first pregnancy as a risk factor	Postponement of the first delivery after age 24 increases a woman's breast cancer risk
	First birth between 30 and 34 years of age is associated with a risk similar to that observed in nulliparous women
	First birth after 35 years of age increases by two third the breast cancer risk
Pregnancy associated breast cancer (PABC)	The hormonal milieu of pregnancy might stimulate the progression of preexisting preneoplastic lesions that are diagnosed during pregnancy or within 1 or 2 years following delivery
	PABC has a worse prognosis and more pronounced mortality than breast cancer diagnosed to women with no PABC
Estrogens and progestagens as carcinogens	17β-Estradiol
	Women with high levels during the first trimester of pregnancy are at increased risk of breast cancer diagnosis before 40 years of age, but decreased in women whose breast cancer was diagnosed after the age 40
	Administration to August/Copenhagen/Irish (ACI) rats induces tumors that are similar to the in situ and invasive ductal carcinomas developed by women
	In vitro it induces neoplastic transformation of the human breast epithelial cells MCF-10F, which become tumorigenic in SCID mice
	Medroxyprogesterone acetate (MPA)
	Administration to BALB/c female mice induces mammary ductal carcinomas in 80% of treated animals. The tumors are ERα and progesterone receptor positive and metastasize to lymph nodes and lung
Hormone replacement therapy (HRT) and postmenopausal breast cancer	In USA and Europe prescriptions for HRT have been linked to 25% of breast cancers (Carsten AJ (2009) S Afr Med J 99:280)
	Breast cancer incidence has decreased following the decline in the use of HRT (Renard et al (2010) Ann Oncol)
	The incidence of lobular cancer increased by 14% in association with the use of HRT (Ravdin PM (2009) Breast Dis 30:3)

1.8 Hormones as Carcinogens

The dependence of breast cancer from estrogens has been demonstrated through the induction of mammary cancer in female August/Copenhagen/Irish (ACI) rats in which administration of 17β-estradiol induces tumors that are similar to the in situ and invasive ductal carcinomas developed by women [72]. Estrogen-induced lesions are completely prevented by concomitant treatment with tamoxifen citrate (TAMc), confirming their estrogen receptor dependence [73]. The carcinogenicity of 17β-estradiol has been confirmed by in vitro experiments through the induction of neoplastic transformation of the human breast epithelial cells MCF-10F, supporting the concept that this hormone could act as an initiator of breast cancer in women [74]. The biphasic effect of estradiol on breast cancer risk is highlighted by the findings that elevated levels of estradiol in maternal serum during the first trimester of pregnancy are positively associated with risk of breast cancer before age 40, but inversely associated with risk in women whose breast cancer was diagnosed after the age 40 [19, 23]. Among the hormones used for contraception, medroxyprogesterone acetate (MPA) also has a biphasic effect when administered to rats of different ages before DMBA inoculation. MPA given for 21 days moderately increases the incidence of adenocarcinomas in 45-day-old rats, and more significantly in 75-day-old rats, whereas the same doses significantly reduced mammary cancer incidence in the 55-day-old rats, and indication that the same hormone and same dose exerts a biphasic effect that is modulated by the age of the rats at the time of initiation of treatment [75]. MPA administered to BALB/c female mice induces mammary ductal carcinomas in 80% of treated animals. The tumors are hormone-dependent ERα and progesterone receptor positive that metastasize to lymph nodes and lungs; the tumors evolve through different stages of hormone dependence that are progesterone receptor dependent [76] (Table 1.3).

1.9 When Does a Full-Term Pregnancy Reduce Breast Cancer Risk?

Pregnancy, which is the gold standard for induction of mammary gland differentiation, needs to be completed for preventing mammary cancer, as demonstrated in rats that their first pregnancy was interrupted 12 days after conception and received DMBA 21 days later [27]. Tumor incidence and number of tumors per animal in pregnancy-interrupted rats and age-matched virgin rats were similar, whereas rats that completed their pregnancy had a significantly reduced tumorigenic response. Completion of the first pregnancy results in full differentiation of the mammary gland that culminates in the secretion of milk, which persists during the length of the lactational period [18, 22]. At postweaning, the lobular structures regress and the cells that remain exhibit a marked reduction in proliferative rate, lengthening in the G_1 phase of the cell cycle, greater capabilities to repair DNA damaged by the

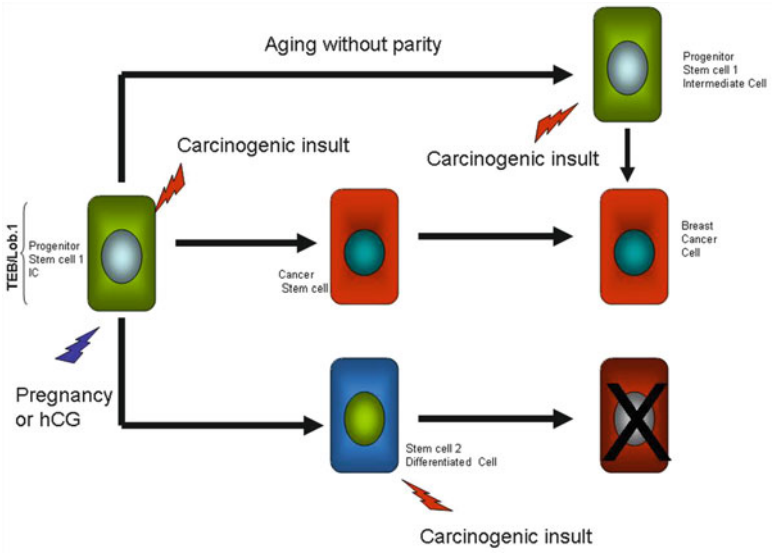

Fig. 1.7 The initially normal progenitor stem cell 1 or intermediate cell (IC) that is present in the TEB/Lob 1 and gives origin to the parenchymal tree, when affected by a carcinogen becomes the cancer stem cell (CSC) that originates breast cancer cells. With aging, in the absence of pregnancy, it remains undifferentiated; if affected by a carcinogen it would retain the capacity of becoming a CSC. Both early pregnancy or hCG treatment of virgin animals induce the differentiation of the progenitor stem cell 1 (IC) to the stem cell 2, which is resistant to be transformed by a carcinogen, although it retains the proliferative activity and capability to regenerate the complete lobular system during the next pregnancy (from Russo IH, Russo J (2011) Pregnancy-induced changes in breast cancer risk. A review. J Mammary Gland Biol Neoplasia 16:221–233)

carcinogen and lower affinity for binding DMBA to DNA [22]. These structural, functional, and molecular changes persist in the mammary gland, resulting in a significant reduction in mammary cancer incidence that is evident in various strains of rats and mice [35], in spite of histopathological differences in tumor type between these species. Blakely et al. [33] have confirmed that in four genetically distinct inbred strains of rats (Lewis, Wistar-Furth, Fischer 344, and Copenhagen) and in mice pregnancy and lactation induce similar structural and genomic changes in mammary glands studied by microarray analysis. Gene analysis identified a genomic signature that sufficed for distinguishing nulliparous from parous animals and explain the almost total refractoriness of the parous rat mammary gland to develop carcinomas after carcinogen administration [33, 77]. These observations indicate that when the development of the mammary gland has been completed by an early pregnancy, steroid hormone- or hCG treatment of virgin animals the PMSC or Stem Cell 1 has completed a first cycle of differentiation under specific hormonal influences, becoming a Stem Cell 2, which is resistant to be transformed by a carcinogen (Fig. 1.7). Although more differentiated, the Stem Cell 2 has retained the capacity to regenerate the complete lobular system required by subsequent pregnancies. This concept has been further demonstrated in transgenic WAP-driven Cre and

Rosa 26-fl-stop-fl-LacZ mice in which parity-induced mammary epithelial cells (PI-MEC) originated from differentiated cells during pregnancy, survived post lactational involution and increased their percentage with successive pregnancies [78]. PI-MEC, like the Stem Cell 2 in the parous rat mammary gland, show capacity for self-renewal and contribute to mammary outgrowth in transplantation studies. PI-MEC can function as alveolar progenitors in subsequent pregnancies, and it is thought that they would be related to differences in response to hormonal stimulation and carcinogenic agents observed between nulliparous and parous females [28, 79, 80] (Table 1.3).

The relevance of the findings that the first full-term pregnancy occurring during the HRSW but before exposure to a carcinogen prevents cancer initiation is equivalent to the well demonstrated protective effect of an early first FTP in women. A first FTP initiated approximately 2 weeks after carcinogen exposure, on the other hand, results in a high incidence of mammary cancer, a phenomenon that could explain the increased cancer risk observed in women first parous after age 30, supporting the assumption that during that lengthened HRSW the breast had been exposed to carcinogenic stimuli before pregnancy. These data emphasize the importance of discriminating whether the first pregnancy would produce protection by inducing complete differentiation of the breast activating the same mechanisms that hormonal treatments do, or would increase breast cancer risk as a consequence of genotoxic or epigenetic exposures during the HRSW (Fig. 1.3).

1.10 The Human Breast in Pregnancy and Disease

The development of the breast is a continuous process initiated by the fourth week of intrauterine life that progresses under the influence of maternal, placental, and environmental factors until birth and by diet and environmental exposures after weaning, respectively. During these periods the maturation of the HPG axis [17, 18, 81] and endogenous hormone secretions play essential roles on the development of the breast at puberty, which is driven by the initiation of ovulation and the establishment of regular menstrual cycles [82]. The architecture of the breast of normally cycling women has been widely described as composed of three main lobular structures that are classified on the basis of their degree of development into lobules type 1 (Lob 1), lobules type 2 (Lob 2), and lobules type 3 (Lob 3) [22, 83, 84] (Fig. 1.8). The breast of women that never conceived a child remains composed of Lob 1, with moderate formation of Lob 2 with successive menstrual cycles (Fig. 1.9); Lob 3 is present only occasionally during the early reproductive years. After menopause the breast further regresses, resulting in an increase in the number of Lob 1 in response to the decline in Lob 2 and Lob 3 with aging (Fig. 1.10). We postulate that the breast parenchyma of postmenopausal nulliparous women contains predominantly Stem Cells 1, which did not achieve the most differentiated stage of Stem Cell 2 due to the absence of pregnancy, therefore retaining its susceptibility to be transformed. Therefore, a carcinogenic insult or an inappropriate hormonal stimulus, such as