Jose Russo

The Pathobiology of Breast Cancer

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Preface

This book is targeted to all those that are wishing to have a comprehensive view of breast cancer and will provide novel information to clinicians, researchers and academia.

This book provides the latest advances in the pathobiology of breast cancer including initiation and progression of the disease, the mechanisms of invasion and metastasis, the concept of stem cells in treatment and drug resistance. The role of personalized medicine and genomic testing provides a window to the future of cancer patient care in diagnosis, prognosis and treatment.

Altogether this book provides a new insight on the pathobiology of the breast using a meticulously researched process that has been lead from basis to translational research.

Philadelphia, PA, USA — Jose Russo, MD, FACP

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Author's Bio

Jose Russo, **MD** is the Director of the Irma H, Russo, MD-Breast Cancer Research Laboratory and Director of the Breast Cancer and The Environmental Research Center at the Fox Chase Cancer Center, Temple Health in Philadelphia, PA. Dr. Russo is also an Adjunct Professor of Pathology and Cell Biology at Jefferson Medical School and Professor of Biochemistry at Temple Medical School. He has received numerous research awards from the National Cancer Institute (NCI) of the National Institute of Health (NIH), from the American Cancer Society and the Department of Defense for his original research in breast cancer. Throughout his career, Russo's work and research interests have had a broad base but with a focused goal: to understand the mechanisms that control cancer metastasis; and to develop strategies for breast cancer prevention.

The original version of this book was revised. An erratum to this book can be found at DOI 10.1007/978-3-319-40815-6_11

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Erratum to: The Pathobiology of Breast Cancer..... E1

Chapter 1
The Windows of Susceptibility to Breast Cancer

1.1 Introduction

The occurrence of cancer of the breast has long been known [1–4] and the disease affects women of all races and nationalities and the incidence of has increased 30–40 % since the 1970s [2, 4–7]. This already dismal picture is worsened by the gradual increase in breast cancer incidence in most Western countries and in societies that recently became westernized or that are in the process of westernization [8, 9]. Epidemiological observations that daughters of women who migrate from low-incidence to high-incidence countries acquire the breast cancer risk prevailing in the new country [10], suggest that aspects of lifestyle or the environment are major determinants of breast cancer risk. A study of population-attributable risks has estimated that at least 45 % to 55 % of breast cancer cases in the United States may be explained by the following factors: advanced age at the time of the first full-term pregnancy, nulliparity, family history of breast cancer, higher socioeconomic status, earlier age at menarche, and prior benign breast disease [11]. Other statistical models appear to explain an even higher proportion of breast cancer on the basis of known risk factors [12]. Studies of atomic bomb survivors have shown that environmental exposures, such as ionizing radiation, are a risk factor for breast cancer [13]. Exposure to radiation at a young age (Fig. 1.1) has been identified as a causative agent of breast cancer in selected populations [14–17], but there is no definitive proof of what causes breast cancer in the population at large. The increased risk associated with exposure to environmental chemicals, such as alcohol [18] and cigarette smoke [19–25], makes these agents suspects for causing cancer in the human population (Fig. 1.1).

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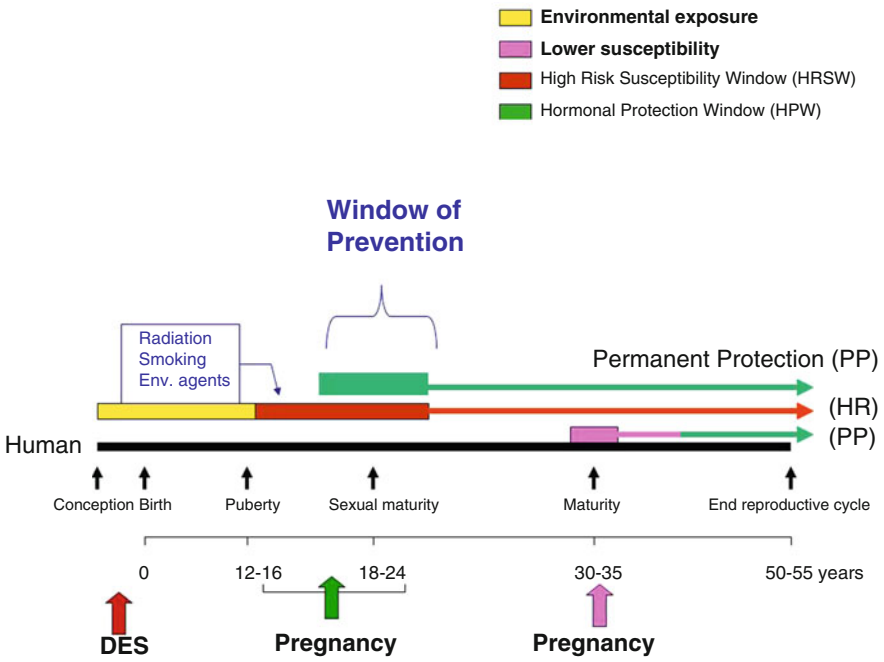

Fig. 1.1 Windows of risk and prevention

1.2 Risk Factor and Etiological Agents

Breast cancer rates among older women have been reported to be higher in the Northeast than in the South; they are also higher in urban than in rural areas [26, 27]. There is an increasing linear relationship between standardized incidence rates of breast cancer and population density [28]. There is a higher breast cancer incidence in the San Francisco Bay Area when compared to the other seven registries located throughout the country [29]. Regional differences in the prevalence of known breast cancer risk factors, such as low parity, higher education, and higher income, seem to play an important role in the elevated rates of breast cancer reported in affluent communities, such as in Marin County, California [30]. In addition some of the geographic variations in breast cancer have been attributed to differences in exposure to sunlight [31–33].

Radiation from natural sources is ubiquitous in the environment [34]. Only accidental or iatrogenic radiation has been demonstrated to exert a carcinogenic effect on the breast. High energy X- or γ rays from a bomb exposure in Hiroshima and Nagasaki in Japan [35], and chest radiation administered for the treatment of scoliosis, or repeated fluoroscopies for tuberculosis [36] have been well-documented causes of breast cancer (Fig. 1.1). Importantly radiation has a carcinogenic effect when exposure occurs at a young age. Only those women who were younger than 29 at the time of the bombing in Japan developed breast cancer [35], whereas older

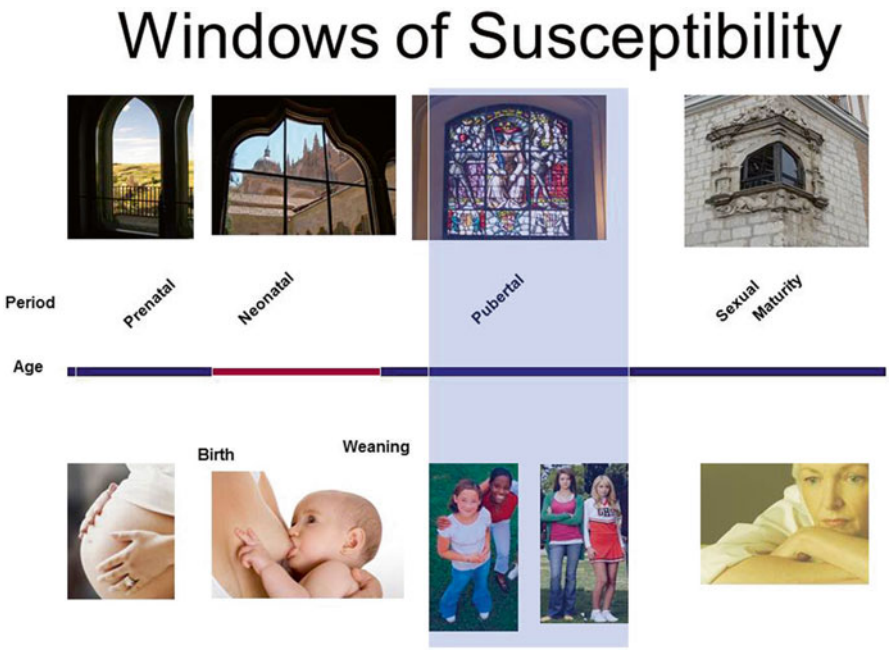

Fig. 1.2 The pubertal period is the windows that offer the highest risk for cancer initiation and is also the best windows for cancer prevention

women developed benign breast diseases. There is evidence that age at exposure influences the relative risk (Figs. 1.1 and 1.2). Excess risk decreases with increasing age at exposure; the highest relative risks are observed for women exposed between the ages of 10 and 20, and there is little risk for those greater than 40 [37–39] (Figs. 1.1 and 1.2). Parity, in addition to age at exposure, modifies the risk of developing radiation-induced breast cancer, since the risk is greater in nulliparous women, but no carcinogenic effect has been reported in women treated with radiation for postpartum mastitis. However, young women that are successfully treated with radiation for early-stage Hodgkin's disease [14–17] develop breast cancers after a median interval of 15 years. While the risk of recurrent Hodgkin's disease decreases as time from treatment elapses, the risk of radiation-induced breast cancer rises. Women irradiated between the time of puberty and the ages of 30 are at the highest risk of developing cancer. It would be of great interest to consider the possibility of preventing breast cancer in this patient population by administering a hormonal treatment that would differentiate their breast "before" or shortly after administering radiation, in order to reduce the susceptibility of the organ to be transformed by the treatment (Fig. 1.3).

Exposure to environmental lighting in the visible range of the spectrum [40] and low-level EMF [41] have been hypothesized to increase the risk of breast cancer due to a decrease in the secretion of the hormone melatonin and a subsequent increase in circulating estrogens [42–48]. The general population is exposed to EMF

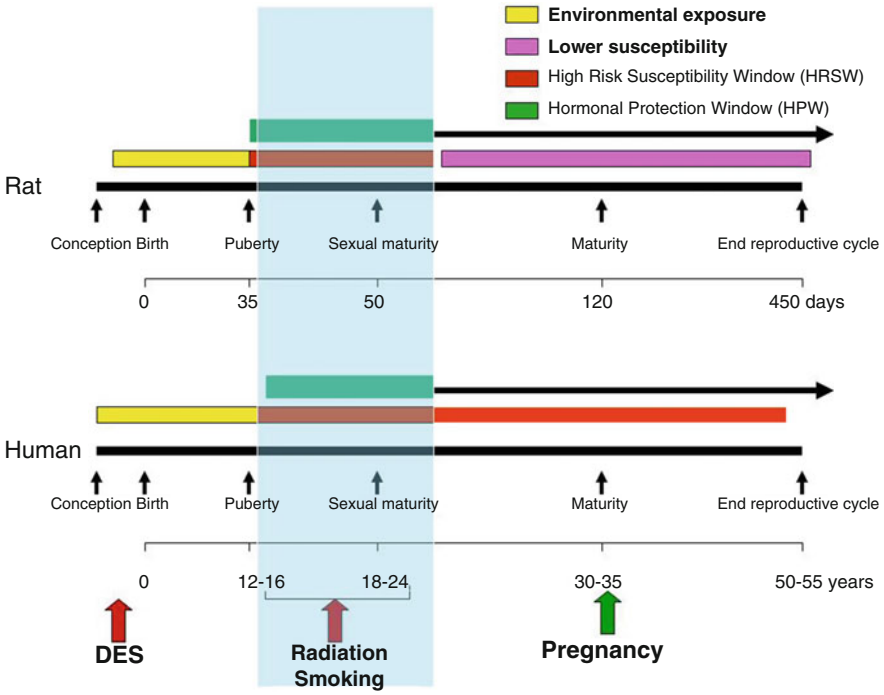

Fig. 1.3 Comparative windows of susceptibility for the rat mammary gland and the human breast

primarily from power lines, transformer substations, and electrical appliance use. Elevation in female breast cancer incidence has been associated with magnitude of exposure at the current residence [49–52].

Exposure to pesticides, e.g., 2,2-bis(p-chlorophenyl)-1,1,1-trichloromethane (DDT), chlordane, hexachloro-cyclohexane (HCH, lindane), hexachlorobenzene (HCB), kepone, and mirex; industrial chemicals, e.g., polychlorinated biphenyls (PCBs); and dioxins (polychlorinated dibenzo-furans (PCDFs), and polychlorinated dibenzodioxin (PCDDs), produced as combustion byproducts of PCBs or contaminants of pesticides have been postulated to increase the incidence of breast cancer . Most of the recent large studies, however, have not found evidence of increased breast cancer risk associated with blood levels of DDE or total PCBs. The possibility exists that a positive association might be limited to women with particular reproductive characteristics [53]. Exposure of the general population to environmental compounds occurs predominantly through ingestion of fish, dairy products, and meat [54]. The experimental and epidemiological evidence of potential links to cancer has been reviewed in detail elsewhere by Adami et al. [55], Ahlborg et al. [56], and Wolff and associates [57].

All women in the general population are exposed to similar environmental influences; yet, not all of them develop breast cancer. Among women with no family history of breast or breast/ovarian cancer [58, 59], or Li-Fraumeni

Syndrome [60], the higher risk of developing breast cancer is associated with a history of early menarche [61], nulliparity [62–65], late first full-term pregnancy [62, 66], and late menopause [62] (Figs. 1.1 and 1.3), all conditions that are under the direct control of the ovary. The central role played by the ovary in breast cancer development is further confirmed by the marked reduction in cancer incidence after surgical or chemical ovariectomy [67]. The indirect evidence that depression of gonadal function, attributed to elevated melatonin levels in profoundly blind women, decreases the risk of breast and other cancers [68–71] suggests that light acts as an important environmental factor modulating breast cancer risk through endocrine disruption [72]. The paradox that ovarian stimulation such as that induced by pregnancy [58–62] or by treatment of women with the pregnancy hormone human chorionic gonadotropin (hCG) [73] exerts a protective effect, highlights the importance of induction of complete breast differentiation for protecting the breast from developing cancer (Fig. 1.3). Differentiation, however, has to be induced during a specific period in the lifetime of a woman, as indicated by epidemiological observations that a full-term pregnancy that markedly reduces the lifetime breast cancer risk of a woman if it occurs before 24 years of age, increases the risk above that observed in nulliparous women when it is postponed beyond the 30th to 35th birthday [62–66] (Figs. 1.1 and 1.2).

1.3 The Concept of the Windows of Susceptibility to Carcinogenesis

Spontaneous mammary tumors are frequently observed in long term rodent studies [74, 75]. 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) [76] and the polycyclic aromatic hydrocarbon (PAH) 7,12-dimethylbenz(a)anthracene (DMBA) [77], or the alkylating agent N-methyl-N-nitrosourea (MNU) [78, 79]. 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 [80]. 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 [80]. The majority of rat mammary tumors induced by DMBA or MNU are ductal adenocarcinomas that are ER-α positive and reproduce the pathological features of the most frequent type of adenocarcinomas developed by women [81]. 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 [74, 82, 83].

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 virgin rats induces maximal tumorigenic response [5–7, 84–93] (Fig. 1.3). 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 (Figs. 1.1, 1.2 and 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 [94–97] (Fig. 1.3). The peak of cancer incidence occurring when virgin rats reach the age of 45 to 55 days and have had at least two ovulatory cycles after vaginal opening [98], 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 [74, 99]. PMSCs that have been primed by ovarian hormones for the expansion the mammary parenchyma through branching and lobular formation, when they are exposed to a carcinogen, such as tritiated (^{3}H) DMBA they exhibit the highest rate of carcinogen uptake as well as a high rate of cell proliferation [74]. Within a few days transformed PMSCs expand and form intraductal proliferations (IDPs) that 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 [99, 100]. Morphologically similar cells have been isolated from DMBA-induced mammary tumors [101]. In the mouse the mammary gland continually undergoes postnatal developmental changes that are driven by signals from TEBs [102]. 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 [102].

1.4 The Windows of Susceptibility Apply to Human Breast Cancer

The comparison of events influencing the initiation of cancer in humans and animals and of the factors that influence both led us to postulate that a commonality exist. Central to this hypothesis is the initiation of puberty (Figs. 1.1 and 1.2). Menarche, or the first menstruation that marks the initiation of puberty, is an objective manifestation of ovarian function [69, 103]. The age at menarche has been observed to decrease in the Western world, with no clear explanation for this phenomenon. It is of great importance to take into consideration the facts that pubertal development is modulated by ovarian function, which in turn is under the control of the hypothalamic-pituitary

axis under the control of two interacting timekeeping mechanisms in the central nervous system (CNS): endogenous circadian rhythmicity and sleep-wake homeostasis [72]. Circadian rhythmicity is an endogenous, near 24-h oscillation, generated in the suprachiasmatic nuclei (SCN) of the hypothalamus (H) that generate pulses transmitted to the pituitary-gonadal (PG) axis via neural and humoral mechanisms. The SCN, under the light-dark cycle, controls the pineal gland and the levels of circulating melatonin. The photoperiod via melatonin secretion determines the timing of puberty in some species and delays reproductive maturity in both males and females. The production of melatonin is inhibited by visible light, which alters the circadian rhythm, disrupting the body's physiology and metabolism [72]. The stimulus of the ovary by pituitary follicle stimulating hormone (FSH) results in follicular maturation and estrogen secretion, followed by a mid-cycle peak of luteinizing hormone (LH) that triggers ovulation and subsequent progesterone secretion. Ovarian stimulation per se is insufficient for driving the breast to the completely differentiated condition that should be reached for achieving protection from cancer development. Additional hormonal supplementation, such as that provided by full-term pregnancy, or specific hormonal regimens, are required for that purpose [73] (Fig. 1.3).

In the last two decades, approximately 3 million women have died prematurely from smoking-related diseases, including cancer. In 1998, 22 % of all women in the USA smoked cigarettes, with a higher percentage of high school senior girls smoking (Figs. 1.1 and 1.3). Lung cancer surpassed breast cancer as the leading cause of cancer death in women and it killed nearly 68,000 women in 2000 [20, 104]. Tobacco smoke is a complex mixture of several thousand chemicals that include carcinogens, namely polycyclic hydrocarbons (PAHs) such as benzo(a)pyrene (BP), which are metabolically activated, forming carcinogen-DNA adducts in human breast tissues. BP selectively binds to deoxyguanine at CpG dinucleotides within codons of the gene, making them mutational hotspots. The resultant G:C to T:A transversions in the p53 gene show a dose-response relationship in lung cancers of smokers [20, 104]. The need to determine whether tobacco smoking is a causative agent in breast cancer has stimulated numerous studies at both epidemiological and basic research levels. The fact that several studies support the hypothesis that "women are more susceptible than men to smoking-induced lung cancer," and that estradiol regulates activities that enhance lung carcinogenesis and tumor progression, as supported by the detection of ER and progesterone receptors (PR) in lung cancer [104], indicate that there similarities at least in hormone dependence between breast and lung cancer. The use of smokeless tobacco has been reported to elevate significantly the risk of developing younger-onset (<55 years) breast cancer (OR = 7.79, 95 % CI = 1.05--66.0) for ever-users of smokeless tobacco [19]. Additional support to the etiologic role of chemical carcinogens in the initiation of breast cancer has been obtained from experimental studies of in vitro transformation of human breast epithelial cells with BP [105].

A clear association has been found between breast cancer occurrence and drinking alcohol. Any history of drinking alcohol increases the risk of breast cancer 1.2-fold above that of women who never drank alcohol (95 % confidence interval 0.7--1.8). A greater than average consumption of alcohol for 6 months or

more increases the relative risk of breast cancer to 2.6 (95 % confidence interval 1.1--5.8) [18]. Positive dose-response trends have been also reported in pooled analysis of large cohort studies and meta-analyses of a broader spectrum of studies. Although the ultimate mechanism through which alcohol increases breast cancer risk has not been clarified, it has been suggested that alcohol consumption alters hormonal levels by affecting the opioid hypothalamic activity, altering LH secretion [103]. These changes have been associated with an advance in the onset of sexual behavior when alcohol is consumed before puberty [103] (Fig. 1.2). An effect of alcohol consumption on the hypothalamic-pituitary-gonadal axis is also supported by the reported observations that the nocturnal urinary concentration of 6-sulfatoxymelatonin, the primary metabolite of melatonin, decreases in a dose-dependent manner with increasing consumption of alcoholic beverages in the preceding 24-h period [106]. A categorical analysis revealed no effect of one drink, but a 9 % reduction with two drinks, a 15 % reduction with three drinks, and a 17 % reduction with four or more drinks [106].

A higher breast cancer incidence has been reported in women that work at night, whereas the risk is lower in profound bilateral blind women [69–71]. The rate of breast cancer risk reduction in blind women is proportional to the degree of blindness [72]. Experimental studies have demonstrated that exposure to constant light from birth enhances mammary carcinogenesis in rodents [70]. Pinealectomy, that suppress nocturnal melatonin production and increases prolactin levels, also stimulates tumor progression in rodents [71]. Exposure to constant light initiated at the age of 26 days in rat, on the other hand, induces lactational changes and inhibits rat mammary carcinogenesis. A suggested mechanism for the effect of light on mammary carcinogenesis is its suppressive effect on nocturnal melatonin, in association with increased levels of DNA synthesis and elevated circulating levels of prolactin [70–72]. Discrepancies in results obtained when constant light exposure is initiated at birth and those initiated at 26 days of age might reflect age-related differences in the maturation of the suprachiasmatic nucleus (SCN) and hypothalamic-pituitary-gonadal axis in response to environmental stimuli that deserve further investigation. During the past years, significant progress has been made in identifying the molecular components of the mammalian circadian clock system [107]. An autoregulatory transcriptional feedback loop similar to that described in Drosophila appears to form the core circadian rhythm generating mechanism in mammals. Two basic helix-loop-helix (bHLH) PAS (PER-ARNT-SIM) transcription factors, CLOCK and BMAL1, form the positive elements of the system and drive transcription of three Period and two Cryptochrome genes. The protein products of these genes are components of a negative feedback complex that inhibits CLOCK and BMAL1 to close the circadian loop. The novel findings that environmental carcinogens bind to epithelial cells through the aryl hydrocarbon receptor (AhR), which upon translocation to the nucleus it dimerizes with the co-factor AhR nuclear translocator (ARNT), a member of the Per-ARNT-Sim (PAS) protein-containing the same transcription factors CLOCK and BMAL1 found in the suprachiasmatic nucleus (SCN), suggest that in addition to being involved in the

initiation of puberty, the SCN might be affected by environmental carcinogens that are traditionally considered to exert their carcinogenic effects on peripheral organs by acting directly on mammary epithelial receptors.

1.5 Effect of Hormones on Breast Cancer

The hormone dependence of breast cancer that had been established by Beatson in 1896 [108] was not recognized in laboratory animals until Huggins et al. [76] 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 17-β estradiol increased mammary cancer incidence to 100 %, whereas rats receiving 20 μg 17-β 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 estrogen receptor alpha (ERα), such as tamoxifen [109] 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 [76, 109].

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 [83, 110, 111] (Fig. 1.3). Treatment of various strains of rats with hormonal combinations, i.e., ethinyl estradiol-megestrol acetate; ethinyl estradiol-norethindrone [74, 93], or 17β estradiol-progesterone [112] two 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 [113, 114].

1.6 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 [115]. Estrogen-induced