Molecular and Cellular Basis of the Mammary Gland Susceptibility to Carcinogenesis

by Jose Russo,* Lee K. Tay,* Daniel R. Ciocca* and Irma H. Russo*

Mammary carcinomas induced by the administration of 7,12-dimethylbenz(a)anthracene (DMBA) to young virgin rats arise from undifferentiated terminal ductal structures called terminal end buds (TEBs). TEBs that normally differentiate into alveolar buds (ABs) and lobules under the influence of DMBA develop intraductal proliferations which progress to carcinoma. The high susceptibility of the young virgin rat TEBs to neoplastic transformation is due to its large proliferative compartment, with cells cycling every 10 hr, and to a higher 3H-DMBA uptake. Progressive differentiation of TEBs into ABs and lobules or their regression to terminal ducts (TDs) is seen with aging. Complete differentiation of the gland is attained only through pregnancy and lactation. The greater differentiation of the gland is manifested as permanent structural changes, consisting in the disappearance of TEBs and in a diminution of the number of TDs due to their differentiation into ABs and lobules. This greater differentiation results in a diminished or total refractoriness of the gland to the carcinogen because ABs and lobules have a lower proliferative compartment and a longer cell cycle than TEBs and TDs. Cells of parous rats have both in vivo and in vitro a lower DMBA-DNA binding capacity, a lower DNA synthesis and a greater ability to repair DMBA damaged DNA than cells of young virgin rats. The more efficient DNA repair capacity of the parous rat mammary gland is demonstrated by the induction of unscheduled DNA synthesis and a removal of DMBA-DNA adducts.

Introduction

Breast cancer is a tumor that arises from a hyperplastic growth of the mammary gland epithelium (1-6). Although the etiologic agent(s) which stimulate the epithelial growth that eventually evolves into neoplasia is not yet known, certain common denominators have been found (Table 1) (7-75). Epidemiologic observations reveal that mammary carcinomas occur more frequently in nulliparous than in multiparous women (27-29, 47, 76-78), in women having an early menarche (12-19, 79, 80) and in those whose first pregnancy occurs after 25 years of age (12-15, 81). Pregnancy before age 18 (11, 12, 81, 82) and late menarche (79) are related to a lower risk of breast carcinoma. Just how women are protected by early pregnancy has been widely discussed, but there is very little information beyond the fact itself. The understanding of the mechanism by which early pregnancy protects women from breast cancer requires a thorough comprehension of the pathogenesis of the disease and how the reproductive history influences the susceptibility of the mammary gland to carcinogenesis. Due to sampling and ethical limitations intrinsic to human experimentation, what is needed is an experimental model for breast tumors which mimics the most significant aspects of the human disease. Recent studies (62, 83-86) suggest that

Table 1. Common denominators in the natural history of breast carcinoma.

| Common Denominator                        | Reference |
|-------------------------------------------|-----------|
| Endocrine or hormone-related factors      |           |
| Age at menarche, first pregnancy, and menopause | (7-75)    |
| Parity                                    |           |
| Androgen and estrogen secretion           |           |
| Anovulatory cycles                        |           |
| Environmental factors                     |           |
| Geography                                 |           |
| Diet                                      |           |
| Socioeconomic status                      |           |
| Familial and/or heredity factors          |           |
| Familial aggregation                      |           |
| Specific antigens                         |           |
| Cerumen type                              |           |

*Department of Pathology, Michigan Cancer Foundation, 110 E. Warren Avenue, Detroit, MI 48201.
mammary carcinomas induced in Sprague-Dawley rats by 7,12-dimethylbenz(a)anthracene (DMBA) constitutes such a model. DMBA-induced rat mammary carcinomas are hormone-dependent adenocarcinomas histologically similar to human breast tumors (62, 87, 88). Tumor incidence is higher when the carcinogen is administered to nulliparous rats (62, 83, 89). Although tumor growth is stimulated by pregnancy (88, 90), a full-term pregnancy and lactation prior to carcinogen administration inhibits tumor development (83, 85). Therefore, the relationship between nulliparity and the probability of the female rat of developing mammary cancer after carcinogen administration is similar to that observed in human females.

Other carcinogens such as N-methyl-N-nitroso-urea (91) and aromatic amine derivatives have also been used to induce mammary carcinomas (92, 93). However, the model system most used for the study of the pathogenesis of the disease as well as the understanding of the mechanism of susceptibility is the DMBA-induced mammary carcinoma rat experimental model. It is likely that the knowledge gained with this experimental model could also be applied to mammary cancers induced by other carcinogens and ultimately for the understanding of the human disease.

**Induction of Mammary Tumors by Polycyclic Aromatic Hydrocarbons (PAH)**

Many PAHs are potent carcinogens and mutagens that are widely distributed as pollutants in the environment. Of great significance in breast carcinogenesis is the finding of Huggins and co-workers (94, 95) that under optimal conditions, intragastric instillation of DMBA or benzo(a)pyrene (B(a)P) to rats induces mammary cancers. Mammary cancers have also been detected when PAH are administered by other means (96, 97); they have also been regarded

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**Figure 1.** Postnatal development of the rat mammary gland and site of origin of DMBA-induced mammary carcinoma: (a) terminal end buds at 50 days of age; (b) TEBs differentiated into alveolar buds; (c) alveolar buds differentiated in the small ductules of the virginal lobules; (b') intraductal proliferation originated from TEB after DMBA treatment; (c') microtumor.
as direct-acting carcinogens because microgram quantities are capable of causing cancer at the site of administration (98).

Pathogenesis of Rat Mammary Carcinomas

DMBA administration to young virgin rats induces a high incidence of carcinomas. This is due to the fact that the mammary gland of young virgin animals is in an early stage of development and is composed of numerous terminal end buds (TEBs) which are actively differentiating into alveolar buds (ABs) (Fig. 1). TEBs (Fig. 1a) present in the mammary gland at the time of carcinogen administration are transformed by DMBA, becoming larger due to intraductal proliferation (IDP) of the lining epithelium (Fig. 1b and Fig. 2). IDPs increase progressively in size and become confluent, leading to the formation of microtumors (Fig. 1c) which histologically are adenocarcinomas (62, 87, 99, 100). Although the differentiation of TEBs into ABs is inhibited by DMBA treatment, not all the TEBs present in the mammary gland at the time of DMBA administration progress to IDPs. Some of them differentiate into ABs and occasional lobular development is observed. Those TEBs that are already differentiated into ABs prior to DMBA administration do not develop carcinomas. Most of them either remain unmodified or proliferate moderately, forming microscopic adenomas, or undergo dilatation of the lumen, giving rise to hyperplastic alveolar nodules (HAN) and cysts (Fig. 3). These observations indicate that the carcinogen alters the normal process of differentiation of the gland. The carcinogen requires an adequate structural target that determines the type of lesion induced depending upon the area of the mammary gland with which it is in contact. Thus, the more differentiated the structure at the time of carcinogen administration, the more benign and organized is the lesion which develops (Fig. 3) (101, 102).

Cell of Origin of Mammary Carcinoma

The mammary gland epithelium is composed of three main cell types, intermediate, dark and myo-
epithelial cells, which possess morphological and cell kinetic properties that allow the differentiation of one cell type from another (103, 104). These cells are present in a constant proportion in the various compartments, namely TEB, TD, AB and lobules of the gland (Fig. 4). Following DMBA administration, the distribution of dark and intermediate cells changes in the TD and TEB, with a significant increase in the number of intermediate cells at the expense of dark cells. Myoepithelial cells (MC) are unaffected. The proportion of intermediate cells continues to increase with tumor age and in the well-developed tumor they represent nearly 90% of the total number of cells, while dark cells are reduced to approximately 10%, and MC become indistinguishable (Fig. 4). The intermediate cell type seems to be the site of neoplastic proliferation, as suggested by its higher DNA-LI after DMBA administration, which has been shown to depress the DNA-LI of other cell types, resulting in a progressive increase of intermediate cells during the carcinogenetic process.

**Mammary Gland Differentiation as a Determinant of Susceptibility to Carcinogenesis**

Differentiation is considered to be the process by which a cell or a structure advances from an immature to a mature or specialized state. In the case of the rat mammary gland, the sequence TEB→AB→lobules, marks the process of differentiation. The

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**Figure 4.** Effect of DMBA on the distribution of cell population of the TEB and TD of mammary glands. Adapted from Russo, Tait, and Russo (105).

**Figure 5.** Incidence of adenocarcinomas and benign lesions in Sprague-Dawley rats treated at different ages with DMBA. Adapted from Russo, Wilgus and Russo (102) with permission of the editor.
highest density of TEBs (TEBs/mm²) is found in the mammary gland of 21-day-old rats and decreases steadily until the rats reach 63 days of age (101, 102). This decrease is accompanied by the differentiation of this structure into ABs and of these into lobules. The administration of DMBA to rats of different ages induces tumors with an incidence which is directly proportional to the density of TEBs which are ready to differentiate into ABs. The highest incidence of carcinomas and number of tumors per animal is obtained when DMBA is administered to rats which are 40 and 46 days old, a period when TEBs are most actively differentiating into ABs (Fig. 5).

The susceptibility of the mammary gland to carcinogenesis decreases significantly with age (102, 106). This has been explained as a consequence of a diminution in the number of undifferentiated structures. The sharp decrease in TEBs/mm² observed in animals older than 55 days is also accompanied by a lower incidence of tumor formation as well as a lower number of tumors per animal with increasing age (101, 102). ABs which are more differentiated structures are the site of origin of benign lesions, the number of which increases with aging as a consequence of the greater density of ABs present in the gland when DMBA is administered at older ages (Fig. 5) (101, 102).

Mammary tumorigenesis by carcinogens is also inhibited in rats in which mammary growth has been prestimulated by hypothalamic lesions (107), or pituitary grafting (108) or when the carcinogen is administered to lactating rats (89). Decreased tumor incidence has also been observed when mice (109) and rats (90, 106, 110) are inoculated with chemical carcinogens after pregnancy and lactation. The protective effect of pregnancy and lactation that extends to the postweaning period has been attributed to the higher degree of differentiation of the mammary gland (101). This protective effect is manifested by a significantly low incidence of carcinomas developed by parous rats when a single dose of DMBA is administered after the glands have regressed to a resting stage (Table 2). This indicates that it is not the hormonal status of pregnancy and lactation that protect the gland, but the permanent changes induced in the gland structure and in the biological properties of the gland epithelium which are independent of the hormonal status of the host (111). In order to be protective pregnancy must be completed. Pregnancy interruption slightly increases the incidence of carcinomas, and this is related to an incomplete differentiation of the gland (Fig. 6) (112).

### Susceptibility of the Mammary Gland to Carcinogenesis as a Function of Differentiation and Cell Kinetics

The degree of differentiation of the mammary gland can be described at a given time in the life span of the rat according to the number of TEBs, TDs, ABs and lobules. A decrease in the density of TEBs is observed with aging, and in the glands of parous rats, TEBs are undetectable (101, 102, 106). The high susceptibility of TEBs to carcinogenesis is the result of certain characteristics of its epithelium, mainly a high proliferative activity, reflected

![Graph showing incidence of mammary tumors](image)

#### Table 2. Tumor incidence and tumor types developed in rat mammary glands after DMBA administration.

| Group            | No. of rats with tumor/ no. of rats | Percent of rats with tumor | Total no. of tumors | Carcinomas | Fibroadenomas | Latency period, wk. |
|------------------|-------------------------------------|---------------------------|---------------------|------------|---------------|---------------------|
| Young virgin     | 16/21                               | 76.2                      | 34                  | 34         | 100           | 0                   | 8-9                |
| Old virgin       | 14/26                               | 53.8                      | 19                  | 12         | 63            | 7                   | 11-21              |
| Multiparous      | 18/51                               | 35.3                      | 19                  | 4          | 21            | 15                  | 13-19              |

*Data from Russo and Russo (106).*
in high mitotic and DNA synthetic indices (Table 3) (106). The influence of these factors has been substantiated by determining the length of each one of the phases of the cell cycle ($T_c$) and the growth fraction (GF) of the epithelium lining the various terminal structures of the mammary gland (113).

The TEB possesses the highest mitotic and DNA synthetic activities and GF (113), and its epithelium has a very short $T_c$ (Fig. 7). The progressive differentiation of TEB to AB and lobule is accompanied by a diminution in mitotic and DNA synthetic activities and GF, while $T_c$ lengthens, mainly due to lengthening of the $G_1$ phase (Fig. 7). These changes resulting from differentiation are observed within a single gland, but they are accentuated with aging (Fig. 7).

Complete differentiation of the mammary gland as a consequence of pregnancy and lactation eliminates the undifferentiated structures, resulting in a concomitant decrease in mitotic and DNA labeling indices, GF and further lengthening of $T_c$ (113). No variations in the length of $S$ phase are observed either with aging or parity or for the different compartments of the gland. Pregnancy, therefore, induces two basic changes in cell kinetic parameters in the mammary gland: one is the increase in the size of the nonproliferative compartment ($G_s$), and the second is the lengthening of $G_1$ phase of the cell cycle (Fig. 7) (113). These cell kinetic changes could explain the refractoriness of the mammary gland to DMBA-induced carcinogenesis. That is, due to the

Table 3. Percentage of TEB, TD and AB-containing labeled cells and percentage of labeled cells in these structures.a

| Group          | TEB % labeled cellsb | % labeled TEB | TD % labeled cellsb | % labeled TD | AB % labeled cellsb | % labeled AB |
|----------------|----------------------|--------------|---------------------|--------------|---------------------|--------------|
| Young virgin   | 34.4 ± 7.6           | 100          | 12.3 ± 5.8          | 70           | 7.9 ± 3.3           | 50           |
| Old virgin     | 14.8 ± 4.7           | 100          | 4.9 ± 3.4           | 28           | 10.9 ± 1.7          | 5            |
| Multiparous    | 0.0 ± 0.0            | 0            | 0.3 ± 0.5           | 8.2          | 0.3 ± 0.05          | 0.9          |

aData from Russo and Russo (106).

bValues are means ± SD of the DNA labeling indexes at the moment of DMBA administration.

![Figure 7](image-url)  
**Figure 7.** Schematic representations of the cell kinetics parameters in the mammary gland of young, old virgin and parous rats. TEB, terminal end buds; TD, terminal ducts; AB, alveolar buds; GFs, growth fraction expressed as the total number of labeled nuclei/100 cells after 5 days of continuous infusion of [3H]thymidine. The diagram of the cell cycle represents the relative length of the various phases of the cycle for each of the structures shown. Adapted from Russo and Russo (113) with permission from the editor.
effect of pregnancy on differentiation of the mammary gland, TEBs and TDs shift all their cells to the formation of ABs and lobules. When the lobular structures regress from their functional activity, the cells present in the postpregnancy and/or postlactational state have entered $G_0$ or a quiescent state. If the cells are treated at this point in vivo with a carcinogen, they are refractory to carcinoma development (106) and in vitro are less susceptible to the effects of DMBA (114, 115).

Endocrinological Milieu and Susceptibility of the Rat Mammary Gland to Carcinogenesis

Since the mammary gland is under hormonal control (116) and the reproductive history affects the risk of mammary cancer, interest has been generated toward the study of the endocrinological status of rats with different susceptibility to carcinogenesis. Previous reports have shown variations in hormonal levels in different strains of rats and mice with varying incidence of mammary tumors (117-120). However, when rats of the same strain but with different susceptibility to carcinogenesis because of variations in age and in reproductive history are compared, it is found that such differences in susceptibility are not related to prolactin (PRL) or estrogen levels at the time of, or after DMBA administration (Figs. 8-10) (121, 122). Several changes have been observed in young virgin (YV), old virgin (OV) and parous (P) rats after DMBA administration; the most conspicuous are: hyperplasia of pituitary PRL cells, high serum PRL levels, nodular hyperplasia of the adrenal cortex, high serum estradiol levels, and lack of adrenal necrosis in all parous rats and in some old virgin rats.

Notwithstanding the modifications in the hormonal milieu observed in susceptible and nonsusceptible rats after DMBA administration, there is not a clear correlation between the hormonal changes induced and the degree of susceptibility of the mammary gland to carcinogenesis, since parous rats that received the inducer (DMBA) and have the same endocrinological milieu as young and old virgin rats do not develop tumors. This supports the importance of the degree of the mammary gland differentiation at the moment of carcinogen administration as a main factor in the different susceptibility of the gland to carcinogenesis (101, 119).

In Vitro Study of Mammary Gland Susceptibility to Carcinogenesis

The methods used for culture and the characterization and identification of mammary epithelial cells...
have been described (115, 123, 124). Differences in the growth pattern of mammary gland epithelial cells in vitro correlate with the observed cell kinetic variations related to the degree of differentiation of the gland. Epithelial cells from the mammary gland of young virgin rats adapt to the culture conditions rapidly, acting as if the cells were in the logarithmic phase of growth prior to plating. The cells from old virgin and parous rats, on the other hand, require a certain time during which the proliferating cells adapt to the culture condition, as evidenced by the presence of a lag phase of cell growth (Table 4). The number of proliferating cells decreases with age and parity, as evidenced by peak DNA synthetic activity. This means that even when normal growth restraints are removed as the cells are cultured in vitro, only certain cells in the population are able to proliferate, which implies that the differences are intrinsic to the epithelial cells and not to host factors (114).

An important difference in the growth curve of young virgin, old virgin and parous rat mammary cells is that the lag phase is lengthened in the two latter groups, which correlates with observations in vivo (113, 126). This initial resting state observed in old virgin and parous rat mammary gland primary cultures results in a lower DMBA-DNA binding (115) and acts as a protective mechanism against the toxic effect of DMBA when the carcinogen is added immediately after plating, which is more pronounced on the cells of young virgin rats (123, 124). DMBA added to cells at the peak of DNA synthesis induces a greater growth inhibition at lower doses than when the carcinogen is added during the first phase of growth. These observations confirm that cultured cells are generally more sensitive if the carcinogen is added at a time when the cells are actively growing and synthesizing DNA (126-132).

**Metabolic Activation of DMBA**

In common with other PAHs, the mutagenic and carcinogenic activities of DMBA are dependent on its conversion by the P-450/P-450 monooxygenase enzyme systems to chemically reactive electrophilic carbonium ions which will react covalently with critical cellular macromolecules especially DNA within the target tissue (133-135). This latter process is now considered to be the most probable initiation event in malignant transformation (133-137). Differences in susceptibility to DMBA have been observed between virgin and parous rats. This raises the possibility that hormones resulting from the

**Table 4. Effect of degree of susceptibility.**

| Parameters of susceptibility                      | Mammary gland degree of susceptibility |
|--------------------------------------------------|---------------------------------------|
|                                                  | High        | Intermediate | Low          | Reference     |
| Morphological differentiation, cell kinetics     | TEB>TD>AB>LOB | TEB<T>AB>LOB | TD<AB<LOB    | (62, 99, 101, 102, 110) |
| MI                                               | 7.03 ± 1.00 | 2.90 ± 2.30  | 0.09 ± 0.16  | (62, 83)      |
| DNA-LI                                           | 34.40 ± 7.60 | 14.80 ± 4.70 | 0.30 ± 0.50  | (63, 110, 113) |
| Tc                                               | 9.93 ± 0.31 | 18.75 ± 0.99 | 49.63 ± 6.86 | (113)         |
| G.F.                                             | 0.55       | 0.19        | 0.0097       | (113)         |
| Lag phase in culture, hr                         | None       | 24          | 36           | (123, 124)    |
| No. of doublings in culture                      | 3.04 ± 0.3 | 3.00 ± 0.14 | 1.50 ± 0.18  | (123, 124)    |
| "H-DMBA uptake, grains/nucleus                   | 6.80 ± 2.60 | 1.30 ± 0.80 | 0.70 ± 0.50  | (115)         |
| DNA repair                                       | 38         | 25          | 20           |              |
| UDS, % of cells                                  | 20.0 ± 2.8 | 37.3 ± 3.0  | 62.5 ± 3.0   | (128)         |
| Adduct removal at 24 hr, %                       | 15         | 7           | 25           | (125)         |
| Carcinoma development                            | High       | Intermediate | Low to none  | (83, 101)     |
| Benign lesion development                        | Intermediate | High      | Low to none  | (83, 101)     |
pregnancy and ensuing lactation could have modified the mammary epithelial cells in their ability to metabolize DMBA to its carcinogenic species, their ability to bind DMBA to DNA and to repair the resulting DMBA-induced DNA damage.

**DMBA-DNA Binding by Rat Mammary Epithelium**

After administration of DMBA to rats in vivo it binds to the DNA of mammary parenchymal cells with a ratio proportional to the rate of cell proliferation (138-140). It has been demonstrated that a correlation exists between the rats' age and level of DNA synthesis, which in turn is related to the amount of DMBA specifically bound to mammary gland DNA and the susceptibility of the gland to carcinogenesis (138-140). However, the observed differences in DMBA-DNA binding are due not only to age but also to the reproductive history of the rat, since there are differences in the amount of DMBA bound to the DNA of mammary epithelial cells derived from virgin and parous rats. It has been observed in vitro that at all time points, the highest level of DMBA binding occurs in young virgin cells, suggesting that they are more susceptible to the effects of increasing amounts of DMBA (115). The largest difference in terms of binding, however, are obtained in 24-hr cultures where binding in young virgin cells is 20-30% higher than in old virgin cells and 35-40% higher than parous cells over a dose range of 0.1-0.4 μg DMBA/mL. This correlates well with the fact that, at 24 hr, both old virgin and parous cells are still in a resting phase, whereas young virgin cells are already in their logarithmic phase of growth (124). This lower binding of DMBA during the lag phase of old virgin and parous cells in vitro, therefore, implies that both the small growth fraction and proliferative compartment in both groups of animals (106) are important factors in determining the eventual carcinogenic response by reducing the capacity of their cells to bind DMBA.

Therefore, young virgin rats, whose mammary glands contain numerous TEBs with a high proliferative activity show the highest uptake of \(^{3}H\)-DMBA into mammary parenchymal cells in vivo as determined by autoradiographic techniques (Table 5). Uptake into TEB epithelium occurs selectively in the nucleus. More differentiated structures, such as ABs, which are the predominant structures in parous rat mammary gland, are characterized by having a low DNA synthetic activity and mitotic index, both of which are associated with a low DMBA uptake (106) and with a low DMBA-DNA binding in vitro (115). The significance of these results, therefore, lies in the relationship between the high nuclear uptake and the higher DNA synthetic activity in the TEBs (Table 6) and the fact that TEBs are the site of origin of mammary carcinomas (Table 6) (Fig. 3).

**Identification of DMBA-DNA Adducts in Rat Mammary Gland**

Identification of DMBA-DNA adducts in the mammary gland with the use of the Sephadex LH-20 chromatographic method developed by Baird and Brookes (141) reveals that the elution volumes of the DMBA-nucleic acid adduct peaks derived from young virgin, old virgin and parous cells are identical, suggesting that cells from the three groups of rats treated with DMBA in vitro generate similar adducts (125) (Fig. 11). These results are in agreement with those reported for the major DMBA-nucleic acid adducts generated in mouse skin (142), rodent embryo cells in culture (143, 144) and in human mammary epithelial cells (145) treated with DMBA. Although our results are consistent with the bay-region theory of polycyclic hydrocarbon carcinogenesis (146) and provide evidence that

| Table 6. Correlation between the uptake of \(^{3}H\)-DMBA and the DNA-LI in the different compartments of the mammary gland. |
|-----------------|-----------------|-------------|
| Structure      | Grain/nucleus   | DNA-LI      |
| 1 TEB          | 6.8 ± 2.6       | 34.4 ± 7.6  |
| 2 TD + ducts   | 13.2 ± 8.8      | 12.3 ± 5.8  |
| 3 AB           | 7.2 ± 5.0       | 7.9 ± 3.3   |

*Student’s t-tests were done. The following comparisons were significantly different: grains/nucleus, 1 vs. 2, p<0.01, 1 vs. 3, p<0.001; DNA-LI, 1 vs. 2, p<0.01, 1 vs. 3, p<0.001, 2 vs. 3, p<0.05.*

**Table 5. Autoradiography of incorporation of \(^{3}H\)-7,12-dimethylbenz[a]anthracene into the rat mammary gland.**

| Structure   | No. of cells | Grains/ cell | Grains/ nucleus | Grains/ cytoplasm | Grains/ lumen |
|-------------|--------------|--------------|-----------------|-------------------|---------------|
| 1 TEB       | 740          | 9.7 ± 2.9    | 6.8 ± 2.6       | 2.7 ± 0.9         | 0.97 ± 0.2    |
| 2 TD + Ducts| 392          | 2.1 ± 1.4    | 1.3 ± 0.8       | 0.8 ± 0.6         | 0.96 ± 0.6    |
| 3 AB        | 345          | 1.0 ± 0.7    | 0.7 ± 0.5       | 0.5 ± 0.3         | 0.40 ± 0.3    |

*Student’s t-tests were done. The following comparisons were significantly different: grains/cell, 1 vs. 2, p<0.01, 1 vs. 3, p<0.001; grains/nucleus, 1 vs. 2, p<0.01, 1 vs. 3, p<0.001; grains/cytoplasm, 1 vs. 2, p<0.01, 1 vs. 3, p<0.01. Labeled DMBA (\(^{3}H\)-DMBA, 1mCi) with 20 mg cold DMBA as a carrier was dissolved in 1 mL sesame oil and given IG to 55-day-old virgin rats. After 24 hr, the mammary glands were removed, fixed in Bouin’s and processed for light microscopy. Paraffin sections were coated with NTB-2 Kodak emulsion and processed for autoradiography.*
DMBA-3,4-dihydrodiol-1,2-oxide is involved in the activation and binding of DMBA to DNA in mammary epithelial cells, the fact that adducts derived from virgin and parous rats, which are known to have different susceptibility to DMBA-induced mammary carcinogenesis, are identical, indicates that the susceptibility of the gland depends upon factors unrelated to the generation of different DNA adducts. It does not, however, rule out the possibility that the different susceptibility may be due to quantitative differences, since we have already demonstrated that the overall level of binding is different between virgin and parous rat mammary epithelial cells treated with DMBA in vitro (115).

**DNA Repair by Rat Mammary Epithelium in Primary Culture**

Carcinogen-induced DNA lesions are subject to repair by various mechanisms. Since repair of damaged DNA in mammalian cells appears to be an important process with regard to cell susceptibility and cell death, the ability of cells to repair damaged DNA will therefore determine the end biological result of the DNA-carcinogen interaction (125, 147-153). Since parous rats are refractory to the carcinogenic effect of DMBA, it has been postulated that their mammary gland epithelium must repair more efficiently the DMBA-induced DNA damage.

The induction of unscheduled DNA synthesis (UDS) in 48-hr cultures of rat mammary epithelial cells following exposure to DMBA, measured by 3H-thymidine incorporation into the nucleus of cells in the presence of hydroxyurea, indicates that induction of UDS is proportional to the dose of DMBA between 0.1 and 1.0 μg/mL. Induction of UDS is 2- to 4-fold higher in parous cells than in old virgin and young virgin cells, indicating a more efficient DNA repair process by cells of the parous rat mammary epithelium (Fig. 12).

Determination of DNA repair by measuring the efficiency of removal of DMBA adducts (125) following the protocol of Dipple and Hayes (154) reveals that in both young virgin and old virgin cells, the excision of adducts is very slow. For young virgin (YV) cells, the values show that at 5 and 18 hr, the quantitative measure of the excision of DMBA-DNA adducts obtained by calculating the time-dependent decrease in specific radioactivity of the less dense DNA peak following centrifugation on alkaline cesium chloride density gradients reveals that only 3% and 9% of the initial adducts are removed, increasing to only 24% at 48 hr. In old virgin cells, no loss of specific activity is detected within the first 18 hr, and the total loss of adducts after 48 hr is only 12% of the initial amount bound. Cells of parous rats, on the other hand, show an 8% loss of specific activity.
within 5 hr. This loss steadily increases up to 38% by 48 hr. When the relationship between the amount of adducts excised at 24 and 48 hr is plotted against the initial extent of binding (Fig. 13), the results demonstrate that parous cells are capable of a greater and more rapid rate of adduct removal than either old virgin or young virgin cells. Thus, the low tumor incidence observed in parous rats may be due not only to a low DMBA-DNA binding, but also to more efficient DNA repair processes which may have arisen as the result of the cellular differentiation induced in the mammary epithelium by pregnancy and lactation.

Conclusions

The results described above indicate that susceptibility of the mammary gland to carcinogenesis is a composite of multifactorial aspects centered in the differentiation of the gland. The degree of differentiation of the gland as determined by morphological development, cell kinetics parameters and behavior in culture determines the affinity of the target to carcinogen-binding to DNA and the extent and proficiency in repair of damaged-DNA. Based upon these parameters it is possible to classify the susceptibility of the mammmary gland to carcinogenesis as high, intermediate and low (Table 4). Thus measurement of one or more of these parameters can indicate the degree of susceptibility of a given mammary tissue to carcinogenesis. Preliminary results in our laboratory suggest that a correlation between human breast tissue and rat mammary gland findings is possible. (155, 156).

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REFERENCES

1. Sandison, A. T. An autopsy study of the adult human breast. Natl. Cancer Inst. Monogr. 8: 1-145 (1982).
2. Cheatle, G. L. Desquamative and dysgenetic epithelial hyperplasia in breast: their situation and characteristics: their likeness to lesions induced by tar. Brit. J. Surg. 13: 509-532 (1926).
3. Dawson, E. K. The genesis and spread of mammary cancer. Ann. Roy. Coll. Surg. London 2: 241-247 (1948).
4. Foote, F. W., and Stewart, F. W. Comparative studies of cancerous versus noncancerous breasts. Ann. Surg. 121: 197-222 (1945).
5. Gallagher, H. S., and Martin, J. E. Early phases in the development of breast cancer. Cancer 24: 1170-1178 (1969).
6. Wellings, S. R., Jensen, H. M., and Marcum, R. G. An at-
las of subgross pathology of the human breast with special reference to possible precancerous lesions. J. Natl. Cancer Inst. 55: 231-273 (1975).
7. Humphrey, L. J. Relationship of benign breast disease to carcinoma of the breast. Surgery 52: 841-846 (1962).
8. Tanaka, Y., and Oota, K. A stereomicroscopic study of the mastopathic human breast. I. Three dimensional structures of abnormal duct evolution and their histologic entity. Virchows Arch. Pathol. Anat. 349: 195-214 (1970).
9. Black, M. M., and Chabon, A. B. In situ carcinoma of the breast. In: Pathology Annual (S. C. Sommers, Ed.), Appleton-Century-Crofts, New York, 1969, pp. 185-210.
10. Kern, W. H. J., and Brooks, R. N. A typical epithelial hyperplasia associated with breast cancer and fibrocystic disease. Cancer 24: 668-675 (1969).
11. MacMahon, B., Cole, P., Liu, M., Lowe, C. R., Mirra, A. P., Ravinhari, B., Salber, E. J., Valoraas, V. G., and Yuasa, S. Age at first birth and breast cancer risk. Bull. WHO 43: 209-221 (1970).
12. Valoraas, V. G., MacMahon, B., Trichopoulos, D., and Polychronopoulou, A. Lactation and reproductive histories of breast cancer patients in greater Athens, 1965-1967. Int. J. Cancer 4: 350-363 (1969).
13. Lim, T. M., Chen, K. P., and MacMahon, B. Epidemiologic characteristics of cancer of the breast in Taiwan. Cancer 27: 1497-1504 (1971).
14. Parnihar, B., MacMahon, B., and Lindtner, J. Epidemiologic features of breast cancer in Slovenia, 1965-1967. Europ. J. Cancer 7: 295-306 (1971).
15. Yuasa, S., and MacMahon, B. Lactation and reproductive histories of breast cancer patients in Tokyo, Japan. Bull. WHO 42: 195-204 (1970).
16. Shapiro, S., Strax, P., and Venet, L. The search for risk factors in breast cancer. Am. J. Public Health 58: 820-835 (1968).
17. Salber, E. J., Trichopoulos, D., and MacMahon, B. Lactation and reproductive histories of breast cancer patients in Boston 1965-1966. J. Natl. Cancer Inst. 43: 1013-1024 (1969).
18. Segi, M., Fukushima, I., and Fujisaku, S. An epidemiological study of cancer in Japan. Gann 48 (Suppl.): 1-63. (1957).
19. Staszewski, J. Age at menarche and breast cancer. J. Natl. Cancer Inst. 47: 935-940 (1971).
20. Ingleby, H., and Gerchon-Cohen, J. Comparative Anatomy, Pathology, and Roentgenology of the Breast. University of Pennsylvania Press, Philadelphia, 1960, pp. 291-309.
21. Klaer, W. Relation of Fibroadenomatosis (Chronic Mastitis) to Cancer of the Breast. Munksgaard, Copenhagen, 1954, pp. 159.
22. Parks, A. G. The micro-anatomy of the breasts. Ann. Roy. Coll. Surg. Engl. 24: 235-251 (1959).
23. Bonser, G. M., Dossett, J. A., and Jull, J. W. Cyclic disease and epithelial proliferation as precancerous conditions in the human breast. In: Experimental Breast Cancer. Charles C. Thomas, Springfield IL, 1971, pp. 316-363.
24. McLaughlin, C. W., Schenken, J. R., and Tamisiea, J. X. A study of precancerous epithelial hyperplasia and noninvasive papillary carcinoma of the breast. Ann. Surg. 159: 735-744 (1961).
25. Mirra, A. P., Cole, P., and MacMahon, B. Breast cancer in an area of high parity: Sao Paulo, Brazil. Cancer Res. 31: 77-83 (1971).
26. Lilienfeld, A. M. The relationship of cancer of the female breast to artificial menopause and marital status. Cancer 9: 927-934 (1956).
27. Logan, W. P. D. Marriage and childbearing in relation to cancer of the breast and uterus. Lancet 2: 1199-1202 (1953).
28. Damon, A. Host factors in cancer of the breast and uterine cervix and corpus. J. Natl. Cancer Inst. 24: 483-516 (1960).
29. Stewart, H. L., Dunham, L. J., Casper, J., Dorn, H. F., Thomas, L. B., Edgecomb, J. H., and Symeonidis, A. Epidemiology of uterine cervix and corpus, breast and ovary in Israel and New York City. J. Natl. Cancer Inst. 37: 1-95 (1966).
30. Sherman, B. M., and Korenmen, S. G. Inadequate corpus luteum function: a pathophysiological interpretation of human breast cancer epidemiology. Cancer 35: 1906-1912 (1974).
31. Bulbrook, R. D., Hayward, J. L., Spicer, C. C., and Thomas, B. S. Urinary steroid excretion of normal women and women with advanced breast cancer. Lancet ii: 1235-1240 (1962).
32. Bulbrook, R. D., Hayward, J. L., and Thomas, B. S. The relation between urinary 17-hydroxycorticosteroids and 11-deoxy-17-oxosteroids and the fate of patients with mastectomy. Lancet ii: 945-947 (1964).
33. Kumaoka, S., Sakauchi, N., Abe, O., Kusama, M., and Takatani, I. Uterine 17-keo-steroid excretion of women with advanced breast cancer. J. Clin. Endocrinol. Metab. 28: 667-672 (1968).
34. Bulbrook, R. D., Hayward, J. L., and Spicer, C. Relation between uterine androgen and corticoid excretion and subsequent breast cancer. Lancet ii: 395-398 (1971).
35. Wade, A. P., Davis, J. C., and Tweedie, M. C. The discriminant function in carcinoma of the breast. Lancet i: 853-857 (1969).
36. Wade, A. P., Davis, J. C., and Tweedie, M. C. Discriminants and breast cancer. Lancet ii: 54 (1969).
37. Kaplan, S. D., and Acheson, R. M. A single etiological hypothesis for breast cancer? J. Chronic Dis. 19: 1221-1230 (1966).
38. Hirayama, T., and Wynder, E. L. A study of the epidemiology of cancer of the breast. Cancer 15: 28-38 (1962).
39. Feinleib, M. Breast cancer and artificial menopause: a cohort study. J. Natl. Cancer Inst. 41: 315-329 (1968).
40. Grattarola, R. The premenstrual endometrial pattern of women with breast cancer. Cancer 17: 1119-1122 (1964).
41. MacMahon, B., and Cole, P. Endocrinology and epidemiology of breast cancer. Cancer 24: 1146-1150 (1969).
42. Levin, M. L., Sheehe, P. R., Graham, S., and Gildewell, O. Lactation and menstrual function as related to cancer of the breast. Am. J. Public Health 54: 580-587 (1964).
43. Lemon, H. M., Wotiz, H. H., Parsons, L., and Mozden, P. J. Reduced estriol excretion in patients with breast cancer prior to endocrine therapy. J. Am. Med. Assoc. 196: 1128-1136 (1966).
44. Lemon, H. M. Endocrine influences on human mammary cancer formation. Cancer 29: 781-790 (1969).
45. Grounroos, M., and Aho, A. J. Estrogen metabolism in postmenopausal women primary and recurrent breast cancer. Europ. J. Cancer 4: 523-527 (1968).
46. Marmorston, J., Crowley, L. G., Myers, S. M., Stern, E., and Hopkins, C. E. Urinary excretion of estrone, estradiol and estriol by patients with breast cancer and benign breast disease. Am. J. Obstet. Gynecol. 92: 460-467 (1965).
47. Wynder, E. L., Bross, I. J., and Hirayama, T. A study of the epidemiology of cancer of the breast. Cancer 13: 559-601 (1960).
48. MacMahon, B., and Feinleib, M. Breast cancer in relation
to nursing and menopausal history. J. Natl. Cancer Inst. 24: 733-753 (1960).

49. Sunkara, P. S., Rao, P. N., and Nishioka, K. Role of putrescine in DNA synthesis and mitosis of mammalian cells. Proc. Am. Assoc. Cancer Res. 18: 84 (1977).

50. McGregor, D. H., Land, C. E., Choi, K., Tokuoka, S., and Liv, P. L. Breast cancer incidence among atomic bomb survivors. Hiroshima and Nagasaki, 1950-69. J. Natl. Cancer Inst. 59: 799-811 (1977).

51. Mackenzie, I. Breast cancer following multiple fluoroscopies. Brit. J. Cancer 19: 1-8 (1965).

52. Myrden, J. A., and Hiltz, J. E. Breast cancer following multiple fluoroscopies during artificial pneumothorax treatment of pulmonary tuberculosis. Can. Med. Assoc. J. 100: 1032-1034 (1969).

53. Boice, J. D., and Monson, R. R. Breast cancer in women after repeated fluoroscopic examination of the chest. J. Natl. Cancer Inst. 59: 823-832 (1977).

54. Higginson, J. The role of geographical pathology in environmental carcinogenesis. In: Twenty-fourth Annual Symposium on Fundamental Cancer Research: Environment and Cancer. Williams and Wilkins, Baltimore, 1972, pp. 69-79.

55. Haenzel, W., and Kurikara, V. Studies of Japanese migrants I. Mortality from cancer and other diseases among Japanese in the United States. J. Natl. Cancer Inst. 40: 43-68 (1968).

56. Commoner, B., Vithayathil, A. J., Dolarra, P., Sahabdra, N., Madyastha, P., and Cuca, G. C. Formation of mutagens in beef and beef extract during cooking. Science 201: 913-916 (1978).

57. Grasso, P., and O’Hare, C. Carcinogens in food. In: Chemical carcinogens. (C. E. Searsles, Ed.), American Chemical Society, Washington, DC, 1976, pp. 701-728.

58. Surgeon General’s Report. Smoking and Health, Government Printing Office, Public Health Service Publication No. 79-50066, Washington, DC, 1979.

59. NAS. Particulate Polycyclic Organic Matter. National Academy of Sciences, Washington, DC, 1972.

60. Petritakis, N. L., Mason, L., Lee, R., Sugimoto, B., Lawson, S., and Catchpool, F. Association of race, age, menopausal status, and cerumen type with breast fluid secretion in nonlactating women, as determined by nipple aspiration. J. Natl. Cancer Inst. 54: 829-833 (1975).

61. Petritakis, N. L. Genetic cerumen type, breast secretory activity, and breast cancer epidemiology. In: Genetics of Human Cancer. (J. S. Mulvihill, R. W. Miller, and J. F. Fraumeni, Jr., Eds.), Raven Press, New York, 1977, pp. 297-300.

62. Russo, J., Saby, J., Isenberg, W., and Russo, I. H. Pathogenesis of mammmary carcinomas induced in rats by 7,12-dimethylbenz(a)anthracene. J. Natl. Cancer Inst. 49: 435-445 (1977).

63. Dabelow, A., Die Milchdruse. In: Handbuch der Mikroskopischen Anatomie des Menschen. Vol. 3, part 3, Haut und Nebenhoden Organs (W. Bargmann, Ed.), Springer-Verlag, Berlin, 1957 pp. 277-485.

64. Boston Collaborative Drug Surveillance Program. Relation between breast cancer and S blood antigen system. Lancet 1: 301-305 (1971).

65. Armstrong, B. Recent trends in breast cancer incidence and mortality in relation to changes in possible risk factors. Int. J. Cancer 17: 204-211 (1976).

66. MacMahon, B. Risk factors for endometrial cancer. Gynecol. Oncol. 2: 122-129 (1974).

67. Li, F. F., and Fraumeni, J. F. Soft tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann. Intern. Med. 71: 747-752 (1969).

68. Anderson, D. E. Some characteristics of familial breast cancer. Cancer 28: 1500-1504 (1971).

69. Morosini, P., Lee, E. G., Jones, M. N. Breast cancer and the S blood group system. Lancet 1: 411-412 (1972).

70. Patel, R., Habal, M. B., and Wilson, R. E. Histocompatibility (HL-A) antigens and cancer of the breast. Association with antigen HL-A7. Am. J. Surg. 124: 314 (1972).

71. Petrakis, N. L. Cerumen genetics and human breast cancer. Science 173: 347-349 (1971).

72. Lilienfeld, A. M. The epidemiology of breast cancer. Cancer Res. 29: 1503-1513 (1969).

73. Post, R. H. Breast cancer, lactation, and genetics. Eugen. Quart. 13: 1-29 (1966).

74. Vakil, D. V., and Morgan, R. W. Etiology of breast cancer. I. Genetic aspects. Can. Med. Assoc. J. 109: 29-32 (1973).

75. Anderson, D. E. A genetic study of human breast cancer. J. Natl. Cancer Inst. 48: 1029-1034 (1972).

76. Mazzaferli, G. F. Rapporti di frequenza fra carcinoma mammario e gravidanza. Riv. Anat. Pathol. Oncol. 17: 243-246 (1960).

77. Bonser, G. M., Dossett, J. A., and Jull, J. W. Human and Experimental Breast Cancer. Charles C. Thomas, Springfileld, IL, 1961, pp. 397-410.

78. Cutler, M. Tumors of the Breast. JB Lippincott, Philadelphia, 1962, pp 148-158.

79. Juret, P., Couette, J. E., Mandard, A. M., Carre, A., De Loezier, T., Brune, D., and Vernhes, J. C. Age and menarche as a prognostic factor in human breast cancer. Eur. J. Cancer 12: 701-704 (1976).

80. Wotiz, H. H., Shane, J. A., Vigersky, R., and Brecher, P. I. The regulatory role of oestriol in the proliferative action of oestriadiol. In: Prognostic Factors in Breast Cancer IA. P. Forest and P. B. Knockler, Eds.), Longman, London, 1968 pp. 368-377.

81. Cole, P., and MacMahon, B. Oestrogen fractions during early reproductive life in the aetioloogy of breast cancer. Lancet 1: 604-606 (1969).

82. Juret, P., Couette, J. E., Brune, D., and Vernhes, J. C. L'age à la première naissance: une variable à la significación simple, double ou triple eu cancerologie mammaire. Bull. Cancer (Paris) 62: 165-174 (1975).

83. Russo, J., Russo, I.H., Ireland, M., and Saby, J. Increased resistance of multiparous rat mammary gland to neoplastic transformation by 7,12-dimethylbenz(a)anthracene. Proc. Am. Assoc. Cancer Res. 18: 149 (1977).

84. Dao, T. L. Carcinogenesis of mammary gland in rat. Progr. Exp. Tumor Res. 5: 157-216 (1964).

85. Moon, R. C. Relationship between previous reproductive history and chemically induced mammary cancer in rats. Int. J. Cancer 4: 312-317 (1969).

86. Huggins, C., Brizziarella, G., and Sutton, H. Rapid induction of mammary carcinoma in the rat and the influence of hormones on the tumors. J. Exptl. Med. 109: 25-54 (1959).

87. Murad, T., and von Haam, E. Studies of mammary carcinoma induced by 7,12-dimethylbenz(a)anthracene administration. Cancer Res. 32: 1404-1415 (1972).

88. McCormick, G. M., and Moon, R. C. Effect of pregnancy and lactation on growth of mammary tumors induced by 7,12-dimethylbenz(a)anthracene (DMBA). Brit. J. Cancer 19: 160-166 (1965).

89. Huggins, C., Grand, L. C., and Brilliante, F. P. Critical significance of breast structure in the induction of mammary cancer in the rat. Proc. Natl. Acad. Sci. (U.S.) 45: 1294-1300 (1959).

90. Dao, T. L., Bock, F. G., and Greiner, M. J. Mammary car-
cinogenesis by 3-methylcholanthrene. II. Inhibitory effect of pregnancy and lactation on tumor induction. J. Natl. Cancer Inst. 25: 991-1003 (1966).

91. Gullino, P. M., Pettigrew, H. M., and Grantham, F. H. N-Nitrosomethyleurea as mammary gland carcinogen in rats. J. Natl. Cancer Inst. 45: 401-404 (1975).

92. Allaben, W. T., Weeks, C. E., Tresp, N. C., Lovie, S. C., Lazear, E. J., and King, C. M. Mammary tumor induction in the rat by N-acetyl-N-2-fluorenyl hydroxalmines: structure activity relationship. Fed. Proc. 37: 1543 (1978).

93. Shirai, T., Fysh, J. M., Lee, M. S., Vaught, J. B., and King, C. M. Relationship of metabolic activation of N-hydroxy-N-acetyl alyamins to biological response in the liver and mammary gland of the female CD rat. Cancer Res. 41: 4346-4353 (1981).

94. Huggins, C., Grand, L. C., and Biglai, P. F. Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. Nature 189: 204-207 (1967).

95. Huggins, C., and Yang, N. C. Induction and extinction of mammary cancer. Science 137: 257-262 (1962).

96. Payne, S. The pathological effects of the intraperitoneal injection of 5,4-benzpyrene into rats and mice. Brit. J. Cancer 12: 65-74 (1958).

97. Pataki, J., and Huggins, C. Molecular site of substrituents of benzalnaphcne related to carcinogenicity. Cancer Res. 29: 506-509 (1969).

98. Sinha, D. K., and Dao, T. L. A direct mechanism of mammary carcinogenesis induced by 7,12-dimethylbenzalnaphcne. J. Natl. Cancer Inst. (U.S.) 53: 841-846 (1974).

99. Haslam, S. Z., and Bern, H. A. Histopathogenesis of 7,12-dimethylbenzalnaphcne induced rat mammary tumors. Proc. Natl. Acad. Sci. 74: 4020-4024 (1977).

100. Medina, D. Mammary tumorigenesis in chemical cancerogen-treated mice. VI. Tumor-producing capacities of mammary dysplasias in BALBiCrg mice. J. Natl. Cancer Inst. 57: 1185-1189 (1976).

101. Russo, I. H., and Russo, J. Developmental stage of the rat mammary gland determinant of its susceptibility to 7,12-dimethylbenzalnaphcne. J. Natl. Cancer Inst. 61: 1439-1449 (1978).

102. Russo, J., Wilgus, G., and Russo, I. H. Susceptibility of the mammary gland to carcinogenesis. I. Differentiation of the mammary gland as determinant of tumor incidence and type of lesion. Am. J. Pathol. 96: 721-736 (1979).

103. Russo, I. H., Ireland, M., Isenberg, W., and Russo, J. Ultrastructural description of three different epithelial cell types in rat mammary gland. Proc. Electron Microscopy Soc. Am. 34: 146-147 (1976).

104. Russo, J., Isenberg, W., Ireland, M., and Russo, I. H. Ultrastructural changes in the mammary epithelial cell population during neoplastic development induced by a chemical carcinogen. Proc. Electron Microscopy Soc. Am. 34: 250-251 (1976).

105. Russo, J., Tail, L., and Russo, I. H. Susceptibility of the mammary gland to carcinogenesis. III. The Cell of origin of mammary carcinoma. Am. J. Pathol., submitted.

106. Russo, J., and Russo, I. H. DNA labeling index and structure of the rat mammary gland as determinant of its susceptibility to carcinogenesis. J. Natl. Cancer Inst. 61: 1451-1459 (1978).

107. Clemens, J. A., Welsch, C. W., and Meites, J. Effects of hypothalamic lesions on incidence and growth of mammary tumors in cancerogen-treated rats. Proc. Soc. Exptl. Biol. Med. 127: 969-972 (1968).

108. Welsch, C. W., Clemens, J. A., and Meites, J. Effects of multiple pituitary homografts or progesterone on 7,12-dimethylbenzalnaphcne-induced tumors in rats. J. Natl. Cancer Inst. 41: 465-471 (1968).

109. Marchant, J. The inhibitory effect of continued lactation on the incidence of chemically-induced breast tumors in mice of the IF strain. Brit. J. Cancer 12: 55-61 (1958).

110. Russo, J. DNA synthesis and terminal end bud density in mammary gland as determinants of susceptibility to carcinogens. Proc. Am. Assoc. Cancer Res. 19: 228 (1978).

111. Ciocca, D. R., and Russo, J. Prolactin levels in susceptible and non-susceptible rats to DMBA carcinogenesis. Proc. 3rd Ann. Cancer Res. Conf. Ohio Valley Lake Erie Assoc. Cancer Centers, Detroit, Michigan, 1980 pp. 27.

112. Russo, J., and Russo, I. H. Susceptibility of the mammary gland to carcinogenesis. II. Pregnancy interruption as a risk factor in tumor incidence. Am. J. Pathol. 100: 497-511 (1980).

113. Russo, J., and Russo, I. H. Influence of differentiation and cell kinetics on the susceptibility of the rat mammary gland to carcinogenesis. Cancer Res. 40: 2677-2687 (1980).

114. Russo, J., Tay, L. K., and Wilgus, G. Effect of 7,12-dimethylbenzalnaphcne (DMBA) on rat mammary epithelial cells in culture. Fed. Proc. 38: 1249 (1979).

115. Tay, L. K., and Russo, J. 7,12-Dimethylbenzalnaphcne-induced DNA binding and repair synthesis in susceptible and nonsusceptible mammary epithelial cells in culture. J. Natl. Cancer Inst. 67: 155-161 (1981).

116. Anderson, R. R. Development and structure of the mammary gland. Endocrinological control. In: Lactation. A Comprehensive Treatise, VoI I. (B. L. Larson, and V. R. Smith, Eds.), Academic Press, New York, 1974 pp. 97-100.

117. Boyns, A. R., Buchan, R., Cole, E. N., Forrest, A. M. P., and Griffiths, K. Basal prolactin blood levels in three strains of rat with differing incidence of 7,12 dimethylbenzalnaphcne induced mammary tumors. Europ. J. Cancer 9: 169-171 (1973).

118. Sinha, Y. N., Selby, F. W., and Vanderlaan, W. P. The natural history of prolactin and GH secretion in mice with high and low incidence of mammary tumors. Endocrinology 94: 757-764 (1974).

119. Hawkins, R. A., Drewitt, D., Freedman, B., Killin, E., Jenner, D. A., and Cameron, E. H. D. Plasma hormone levels and incidence of carcinogen-induced mammary tumors in two strains of rat. Brit. J. Cancer 34: 546-549 (1976).

120. Sinha, Y. N., Vlahakis, G., and Vanderlaan, W. P. Serum, pituitary and urine concentrations of prolactin and growth hormone in eight strains of mice with varying incidence of mammary tumors. Int. J. Cancer 24: 430-437 (1979).

121. Ciocca, D. R., Parente, A., and Russo, J. Endoerinologue milieu and susceptibility of the rat mammary gland to carcinogenesis. Am. J. Pathol. 109: 47-56 (1982).

122. Ciocca, D. R., Parente, A., and Russo, J. Endoerinologue milieu and susceptibility of the rat mammary gland to carcinogenesis. Am. J. Pathol. 109: 47-56 (1982).

123. Russo, J., and Wilgus, G. Growth kinetics of rat mammary gland epithelial cells in culture. In Vitro 15: 35 (1979).

124. Russo, J., Wilgus, G., Tait, L., and Russo, I. H. Influence of age and parity on the susceptibility of rat mammary gland epithelial cells in primary cultures to 7,12-dimethylbenzalnaphcne. In Vitro 17: 877-884 (1981).

125. Tay, L. K., and Russo, J. Formation and removal of 7,12-dimethylbenzalnaphcne-nucleic acid adducts in rat mammary epithelial cells with different susceptibility to carcinogenesis. Carcinogenesis 2: 1327-1333 (1981).
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126. Shunkin, M. B., Gruenewald, M., Thacher, D., and Berger, R. Tritiated thymidine labeling of cells in rats following exposure to 7,12-dimethylbenzanthracene. Cancer Res. 27: 1494-1499 (1967).

127. Tong, C., Paz, M., and Williams, G. M. Cell cycle-specific mutagenesis at the hypoxanthine phosphoribosyltransferase locus in adult rat liver epithelial cells. Proc. Natl. Acad. Sci. (U.S.) 77: 7377-7379 (1980).

128. Russo, J., Tai, L., and Russo, I. H. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res. Treat. 2: 5-7 (1982).

129. Tominaga, T., Mayo, P. L., and Libby, P. R. Effects of 7,12-dimethylbenzanthracene on RNA polymerase in isolated mammary gland cell nuclei. Proc. Soc. Exptl. Biol. Med. 136: 694-697 (1971).

130. Pound, A. W. Carcinogenesis and cell proliferation. N.Z. Med. J. 67: 88-99 (1968).

131. Marquardt, H., Bendick, A., and Phillips, F. S. Binding of [G-H] 7,12-dimethylbenzanthracene and hepatic neoplasia in regenerating rat liver. Chem.-Biol. Interact. 3: 1-11 (1971).

132. Tominaga, T., Libby, P. R., and Tai, L. An early effect of 7,12-dimethylbenzanthracene on rat mammary gland DNA synthesis. Cancer Res. 30: 118-122 (1970).

133. Miller, E. C., and Miller. J. A. Mechanisms of chemical carcinogenesis. Nature of proximate carcinogens and interactions in the macromolecules. Pharm. Rev. 18: 805-838 (1966).

134. Dipple, A., Lawley, D. P., and Brookes, P. Theory of tumor induction by chemical carcinogens, dependence of activity on structure of ultimate carcinogens. Europ. J. Cancer 4: 493-502 (1968).

135. Miller, E. C., and Miller, J. A. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. Cancer 47: 2327-2346 (1981).

136. Heidelberger, C. Chemical carcinogenesis. Ann. Rev. Biochem. 44: 79-121 (1975).

137. Irving, C. C. Interactions of chemical carcinogens with DNA. In: Methods in Cancer Research (H. Busch, Ed.), Academic Press, New York, 1973, pp. 189-244.

138. Prodi, G., Rocchi, P., and Grilli, S. Binding of 7,12-dimethylbenzanthracene and benzo(a)pyrene to nucleic acids and protein of organs in rats. Cancer Res. 30: 1020-1023 (1970).

139. Marquardt, H., Sternberg, S. S., and Phillips, F. S., 7,12-Dimethylbenzanthracene and hepatic neoplasia in regenerating rat liver. Chem.-Biol. Interact. 2: 401-403 (1970).

140. Janss, D. H., and Ben, T. L. Age-related modification of 7,12-dimethylbenzanthracene binding to rat mammary gland DNA. J. Natl. Cancer Inst. 60: 173-177 (1978).

141. Baird, W. M., and Brookes, P. Isolation of the hydrocarbon-mercapturic acid conjugates products from the DNA of mouse embryo cells treated in culture with 7-methylbenzanthracene-H. Cancer Res. 33: 2378-2385 (1973).

142. Cooper, C. S., Ribeiro, O., Hewer, A., Walsh, C., Grover, P. L., and Sims, P. Additional evidence for the involvement of the 3,4-diol-1,2-oxides in the metabolic activation of 7,12-dimethylbenzanthracene in mouse skin. Chem.-Biol. Interact. 29: 357-367 (1986).

143. Dipple, A., and Nebzydoski, J. A. Evidence for the involvement of a diol-epoxide in the binding of 7,12-dimethylbenzanthracene to DNA in cells in culture. Chem.-Biol. Interact. 20: 17-26 (1978).

144. Dipple, A., Tomaszewski, J. E., Moschel, R. C., Bigger, C. A. H., Nebzydoski, J. A., and Egan, M. Comparison of metabolism-mediated binding to DNA of 7-hydroxymethyl-12-methylbenzanthracene and 7,12-dimethylbenzanthracene. Cancer Res. 39: 1154-1158 (1979).

145. Grover, P. L., MacNicoll, A. D., Sims, P., Easty, G. C., and Neville, A. M. Polycyclic hydrocarbon activation and metabolism in epithelial cell aggregates prepared from human mammary tissue. Int. J. Cancer 26: 467-475 (1980).

146. Jerina, D. M., Yagi, H., Lehr, R. E., Thakker, D. R., Schaeffer-Rider, M., Karle, J. M., Levin, W., Wood, A. W., Chang, R. L., and Cooney, A. H. The bay region theory of carcinogenesis by polycyclic aromatic hydrocarbons. In: Polycyclic Hydrocarbons and Cancer. Environment, Chemistry and Metabolism (H. V. Gelboin and P. O. P. Ts'o, Eds.), Vol. 1, Academic Press, New York, 1978, pp. 173-178.

147. Cleaver, J. E. Defective repair replication of DNA in xeroderma pigmentosum. Nature 218: 652-656 (1968).

148. Regan, J. D., Francis, A. A., Dunn, W. C., Hernandez, O., Yagi, H., and Jerina, D. M. Repair of DNA damaged by mutagenic metabolites of benzo(a)pyrene in human cells. Chem.-Biol. Interact. 20: 279-287 (1978).

149. Maher, V. M., Birch, N., Otto, J. R., and McCormick, J. J. Cytoxicity of carcinogenic aromatic amines in normal and xeroderma pigmentosum fibroblasts with different DNA repair capabilities. J. Natl. Cancer Inst. 54: 1287-1294 (1975).

150. Shinohara, K., and Cerutti, P. A. Excision repair of benzo(a)pyrene-deoxyguanosine adducts in baby hamster kidney 21/C13 cells and in secondary mouse embryo fibroblasts C57BL/6J. Proc. Natl. Acad. Sci. (U.S.) 74: 979-983 (1977).

151. Day, R. S., Scudiero, D., and Dimattina, M. Excision repair by human fibroblasts of DNA damaged by 7,10-di-hydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Mut. Res. 50: 383-394 (1978).

152. Ikenaga, M., Takebe, H., and Ishii, Y. Excision repair of DNA base damage in human cells treated with the chemical carcinogen 4-nitroquinoline 1-oxide. Mut. Res. 43: 415-427 (1977).

153. Hefti, R. H., Hazzard, R. M., Lommel, L., Schriner, J. D., Maher, V. M., and McCormick, J. J. A comparison of the DNA binding, cytotoxicity and repair synthesis induced in human fibroblasts by reactive derivatives of aromatic amide carcinogens. Chem.-Biol. Interact. 29: 43-56 (1980).

154. Dipple, A., and Hayes, M. E. Differential excision of carcinogenic hydrocarbon-DNA adducts in mouse embryo cell cultures. Biochem. Biophys. Res. Commun. 91: 1225-1231 (1979).

155. Tay, L. K., Russo, I. H., Miller, J., and Russo, J. Influence on gland differentiation of binding of 7,12-dimethylbenzanthracene (DMBA) to DNA of human breast epithelial cells in primary culture. In Vitro 17: 201 (1981).

156. Russo, J., Calaf, G., Martinez, F., Schroder, R., Tait, L., and Russo, I. H. Age-related variations in growth kinetics of primary human breast cell cultures. In Vitro 16: 42 (1980).