

Genetic Regulation of Mammary Carcinogenesis in the Rat by Susceptibility and Suppressor Genes

by Michael N. Gould* and Rong Zhang*

Rat strains differ in their susceptibilities to mammary carcinogenesis. Strains such as Wistar-Furth are very susceptible to chemically induced carcinogenesis. This phenotype is controlled by autosomally dominant susceptibility genes. Strains such as the Copenhagen are resistant to spontaneous and induced mammary carcinogenesis. This phenotype is controlled by an autosomal dominant gene termed the mammary carcinoma suppressor (*MCS*). Strains with intermediate susceptibility such as the F344 carry neither the *MCS* nor susceptibility genes. Both the *MCS* and susceptibility genes are chiefly active within the mammary parenchyma. Both genes act at carcinogenesis stages beyond carcinogen metabolism and DNA adduction. The *MCS* gene completely inhibits both palpable and microcarcinomas. It does not inhibit focal alveolar hyperplasias. Its gene product acts solely within the mammary epithelial cell in which it is produced. We are currently investigating the interactions of various oncogenes and the *MCS* gene. In addition, efforts are underway to identify and clone this gene.

Introduction

Genetic predisposition to breast cancer is a major risk factor in women. Factors contributing to this predisposition at both the population and molecular genetic levels are poorly understood at present. Progress is, however, being made both through the study of the population genetics of families with high incidences of breast cancer and the cytogenetic and molecular characterization of human breast carcinomas. For example, we recently cytogenetically characterized a large series of untreated human breast carcinomas and found 3 of 44 with clonal karyotypic changes. A young woman with bilateral carcinomas had a 3p deletion in one tumor, while two other tumors had transitions involving chromosome 1 (1). It has been suggested that these sorts of chromosomal changes are associated with the loss of suppressor gene activity or the activation of certain oncogenes (2).

Most of the suppressor genes and oncogenes that have been identified in human tumors have been largely conserved through eukaryotic evolution. Based on this, we are attempting to identify genes that modulate human breast carcinogenesis by characterizing potential homologues in lower mammals. We chose this path of investigation because of the relative ease of genetically

characterizing species that can be both selectively and rapidly bred. The animal model chosen was the rat, with many inbred strains available for study. The rat was chosen over the mouse because *a*) mammary cancers in the rat, like the human but unlike the mouse, lack a major viral etiology, *b*) tumors of the rat, unlike the mouse, are readily induced by chemicals, radiation, or hormonal treatments, and *c*) the rat mammary tumor resembles that of the human in its spectra of hormonal responsiveness. Unfortunately, the rat has a much less characterized genomic map than does the mouse.

Rat Strain Susceptibility

Rat types (outbred) and strains (inbred) differ in their susceptibilities to both spontaneous and induced mammary cancers. We have recently reviewed the susceptibility of many rat strains to spontaneous and induced mammary tumors (3). The susceptibility of a rat strain to spontaneous carcinomas cannot be predicted by its susceptibility to induced cancer. In previously reviewing the incidence of various spontaneous tumors in different rat strains, we found a strong correlation between the propensity of a specific strain to develop prolactin-secreting pituitary tumors and its likelihood to develop mammary carcinomas (3). This association is mechanistically reasonable, since prolactin has been shown to be a promoter of rat mammary carcinogenesis (4,5). Thus, increased susceptibility to spontaneous mammary cancer is probably controlled via an abscopal mechanism related to hyperplastic and neoplastic

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changes in the prolactin-secreting cells of the anterior pituitary. The propensity of a rat to develop pituitary cell transformation is genetically controlled in an additive fashion by approximately three independently segregating genes (6).

In general, rat strains fall into three susceptibility groups for the development of induced mammary carcinomas. The first group has a high incidence of tumors following carcinogen administration (e.g., Wistar-Furth [WF]), while the second is totally resistant (e.g., Copenhagen [Cop]). The third group, which includes the Fischer (F344) rat, has an intermediate susceptibility (Figs. 1 and 2). We determined the genetic basis for these three categories of rats by segregation analysis. WF rats from the high susceptibility group of rat strains were bred to F344 rats from the intermediate susceptibility group of strains. We tested parent strains as well as F_1 , F_2 , and backcross rats for the development of mammary carcinomas following exposure to the mammary carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA). We found that each group of hybrid rats was phenotypically very similar to the more susceptible parent strain, the WF rat (7) (Fig. 1). These data suggested that the susceptibility-conferring factor in the WF rat was controlled by multiple, independently segregating genes. The action of these genes was not additive, in that rats with one or more of these genes had equal susceptibility to DMBA-induced mammary tumors. When various genetic models were fit to the data, we found that the one suggesting three independently segregating genes fit best. Further experiments in which the sexes of the strains in the analysis were reversed suggested that these genes were not sex linked and that maternal factors or genetic imprinting was not a factor. Whether each independently segregating susceptibility-conferring gene is the same or different remains to be determined.

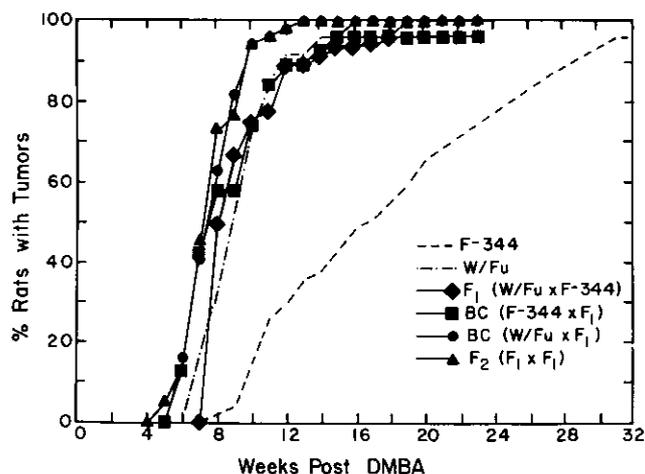


FIGURE 1. Time to first mammary tumor of rats given DMBA (130 mg/kg) at zero time. Rat strains include the parent strains WF and F344 and specific crosses: WF \times F344 F_1 ; backcross F344 \times (F_1); backcross WF \times (F_1); and F_2 ($F_1 \times F_1$, $F_1 = WF \times F344$).

The genetic composition of rats in the second category, which are completely resistant to both induced and spontaneous mammary tumors, was also tested by segregation analysis. Resistant Cop rats were bred with susceptible WF rats. Parent strains, F_1 , F_2 , and backcross hybrid rats were treated with DMBA and followed for the development of mammary carcinomas (Fig. 2). The results of this analysis suggest that the resistance to mammary carcinogenesis found in the Cop rat is due to a single autosomal dominant gene (3). A similar conclusion was reached by Isaacs, who crossed and tested progeny of Cop and Sprague-Dawley rats (8). We have termed this Cop rat gene the mammary carcinoma suppressor or *MCS* gene (9). Thus, we conclude that rats in the high susceptibility category, such as the WF, possess one or more susceptibility-conferring genes, while rats in the most resistant category, such as the Cop, carry a suppressor gene (*MCS*). Rat strains such as F344 that show an intermediate susceptibility to induced mammary cancer contain neither gene. Finally, when animals that contain both genes are bred (e.g., WF \times Cop F_1), the offspring exhibit a resistant phenotype (Fig. 2) (3).

Site of Expression

In order to begin to characterize the function of these genes as well as to develop strategies to clone them, we sought to determine their organ/tissue site of expression and action. We approached this question for both the susceptibility (7) and *MCS* genes (9) by constructing and testing chimeric rats. Chimeric rats are constructed by grafting known numbers of clonogenic mammary cells from donor rats to ectopic sites such as the interscapular white fat pad of recipient rats (10). Here the clonogenic cells divide and redifferentiate to structurally and functionally normal mammary tissue (11). In these assays, the recipient rats are usually F_1 hybrids of two inbred strains. Thus, it is possible to graft tissue from either parent strain into the F_1 recipient rats.

To determine if the susceptibility genes carried by the WF rat are active within the mammary parenchyma or abscopally active such as in the immune or endocrine system, WF-F344 chimerics were constructed and tested. WF \times F344 F_1 hybrids received ectopic mammary grafts from either F344 or WF rats. After ectopic glands differentiated from these transplanted cells, the recipient hybrid rats were given DMBA, and tumors developing in the ectopic sites were followed by weekly palpation. If the WF susceptibility genes were active in host systems such as the endocrine system, then it would be predicted that both F344 and WF ectopic mammary glands should develop equal numbers of tumors since they developed in identical F_1 hosts. On the other hand, if the WF susceptibility genes were active within the mammary parenchyma, then it would be predicted that more carcinomas should develop in the WF ectopic glands. The results supported the latter prediction, in that more tumors developed in the WF ectopic glands when compared to equivalent ectopic F344

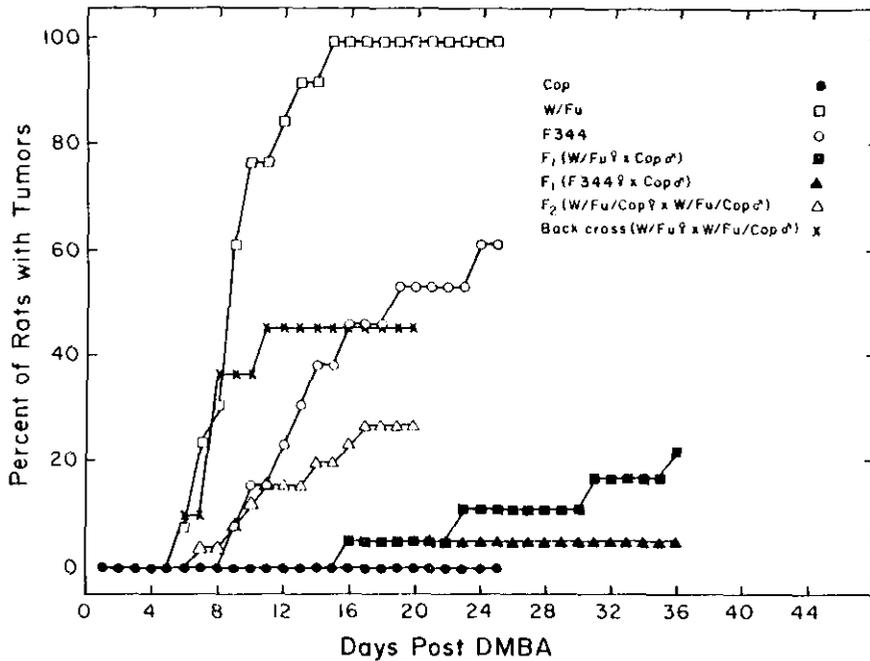


FIGURE 2. Segregation analysis of the gene conferring resistance to mammary carcinogenesis in the Cop rat. Groups of approximately 25 female rats of each parent strain or cross listed in the figure were fed DMBA (130 mg/kg) and followed for tumors. The percentage of rats developing mammary tumors over time is shown in the figure.

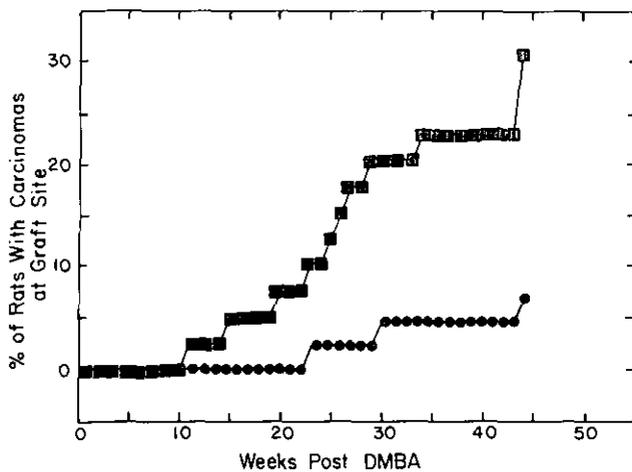


FIGURE 3. Chimeric rats with interscapular grafts of WF (■) or F344 (●) mammary glands were fed DMBA at zero time. The time and percentages of rats that developed mammary carcinomas at the graft sites are plotted.

glands (Fig. 3). This suggests that the WF susceptibility gene mediates its effects within the mammary parenchyma.

A similar approach was taken to identify the site of action of the Cop MCS gene. In an initial experiment, WF cells were grafted into either WF x Cop or WF x F344 recipient rats. These rats were then treated

with DMBA and followed for tumors developing in both grafted and *in situ* mammary glands. This design differed from the one used above to characterize the susceptibility gene because, if Cop cells were grafted into WF x Cop and no tumors arose, one could not determine if this was the effect of the Cop gene in the mammary cell or in the host. It was predicted that if the MCS gene was only active within the mammary parenchyma, then equal numbers of mammary carcinomas would develop in the ectopic WF grafted mammary glands in the two types of hybrid host, even though the WF x F344 hybrid would develop a much greater number of mammary tumors in its *in situ* mammary glands. On the other hand, if the MCS gene was expressed in a nonmammary tissue, such as the endocrine or immune system, then it would be expected that fewer tumors would develop in the WF ectopic mammary gland in the WF x Cop host than in the WF x F344 host. Furthermore, the ratio of tumors in the WF graft sites of these hybrid hosts should be the same as the ratio of mammary carcinomas developing in their respective *in situ* glands. The results obtained were not in complete agreement with either prediction. The ratio of tumors in the *in situ* mammary glands of the WF x F344 versus the WF x Cop hosts was 10:1. The ratio of tumors developing in the WF ectopic glands in these two hosts was 2:1 (Fig. 4). These data suggest that the main suppressor activity associated with the Cop rat resides in the mammary parenchyma. However, a small amount of suppressor activity is also expressed abscopally. This

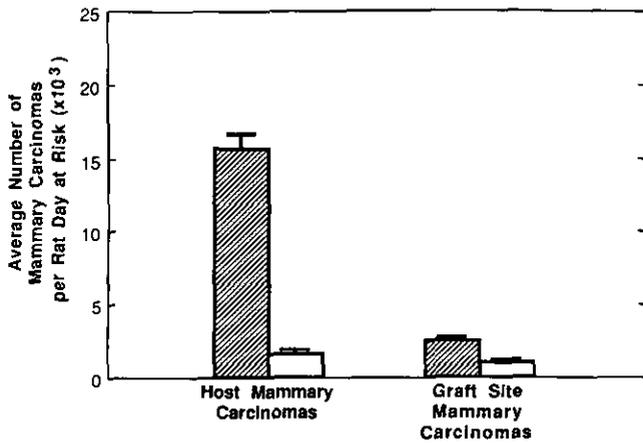


FIGURE 4. Comparison of the ratio of average number of carcinomas in *in situ* and graft site mammary glands. The ratio of average number of host mammary tumors in the WF \times F344 versus WF \times Cop rats is 10 to 1, while at graft sites the ratio of tumors is 2 to 1. Bars represent the mean numbers obtained from each group \pm SE. WF \times F344 (hatched, $n = 99$); WF \times Cop (white, $n = 122$). From Zhang et al. (9).

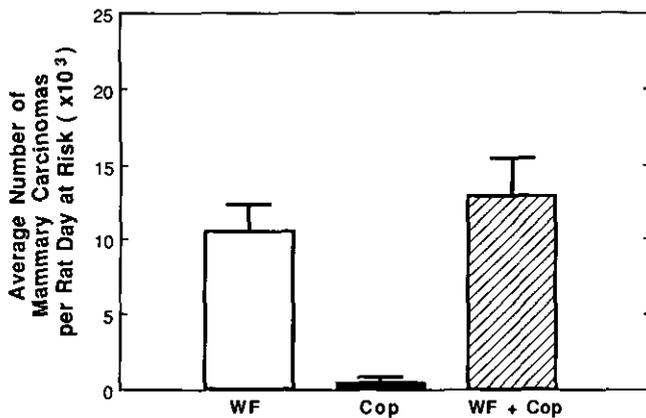


FIGURE 5. Comparison of the mammary carcinomas at ectopic sites grafted with mammary cells from different rat strains. Bars represent the mean numbers of carcinomas of each group \pm SE. WF donor cells grafted into WF \times Cop (white, $n = 122$); Cop donor cells grafted into WF \times Cop (black, $n = 90$); a 1:1 mixture of WF and Cop donor cells grafted into WF \times Cop (hatched, $n = 74$). From Zhang et al. (9).

lesser activity may be due to the expression of the *MCS* gene product at locations outside the mammary parenchyma. Alternatively, it may be due to another Cop gene with a minor suppressor activity that was missed in our segregation analysis due to its low penetrance. We plan to check these alternative explanations by conducting similar experiments in *MCS*-carrying WF congenic rats that are currently being bred.

To confirm that the *MCS* gene was indeed highly active within the mammary epithelium, a second experiment was conducted. Here either Cop or WF ectopic glands were grafted into identical WF \times Cop F₁ hybrid recipients. The numbers of carcinomas arising in the

graft sites were quantitated and compared (Fig. 5). Many tumors developed in the WF glands, while only a single carcinoma was detected in the Cop glands. These data support the above conclusion that the *MCS* gene product is functional within the mammary parenchyma.

Gene Function

The rat *MCS* gene is organ-specific in its action. Dunning and Curtis showed that the chemical carcinogens whose action was suppressed in the Cop mammary gland could cause tumors in other organs such as the liver in this rat strain (12). We extended these observations by investigating the activity of the Cop *MCS* gene in inhibiting various DMBA-induced epithelial pathologies in the rat mammary gland. In the experiments described above, our results were based on the development of palpable carcinomas at the ectopic mammary graft sites. DMBA-exposed graft sites not developing carcinomas were prepared for whole mount observation. Lesions found in the whole mounts were removed and sectioned for histopathological study. Two nonpalpable epithelial lesions were found. These were small mammary carcinomas termed microcarcinomas (Fig. 6) and focal alveolar hyperplasias (FAH) (Fig. 7).

Lesions were compared in DMBA-treated WF ectopic mammary glands growing in WF \times F344 versus those growing in WF \times Cop hybrid recipient rats. We found equal frequencies of microcarcinomas in WF glands in both recipient types (Fig. 8), indicating that the *MCS* gene does not affect microcarcinomas at the host level. However, when Cop cells were grafted into WF \times Cop, no microcarcinomas were found (Fig. 9). Together these data suggest that the *MCS* gene inhibits the development of microcarcinomas and that its action in this regard is totally mediated within the mammary parenchyma (9).

In contrast to the activity of the *MCS* in inhibiting microcarcinomas, we found that this gene had no influence on the development of FAH. We compared the frequency of FAH developing in DMBA-treated ectopic WF and Cop mammary glands growing in WF \times Cop hosts. No statistically significant difference was found in the frequencies of FAH developing in Cop and WF glands (9) (Fig. 9).

We next asked if the *MCS* gene product acted in a paracrine manner, with the *MCS* gene product secreted by the mammary cells into the extracellular space followed by binding of the *MCS* product to mammary cell surface receptors. Such a paracrine mechanism has been demonstrated in human mammary tumors for growth factors such as transforming growth factor α and β (13). This question was approached by establishing a mixed cell mammary gland that contained equal numbers of WF and Cop mammary cells. These mixed glands as well as WF and Cop nonmixed control glands were exposed to DMBA and the development of graft site carcinomas compared. The mixed cell and WF glands did

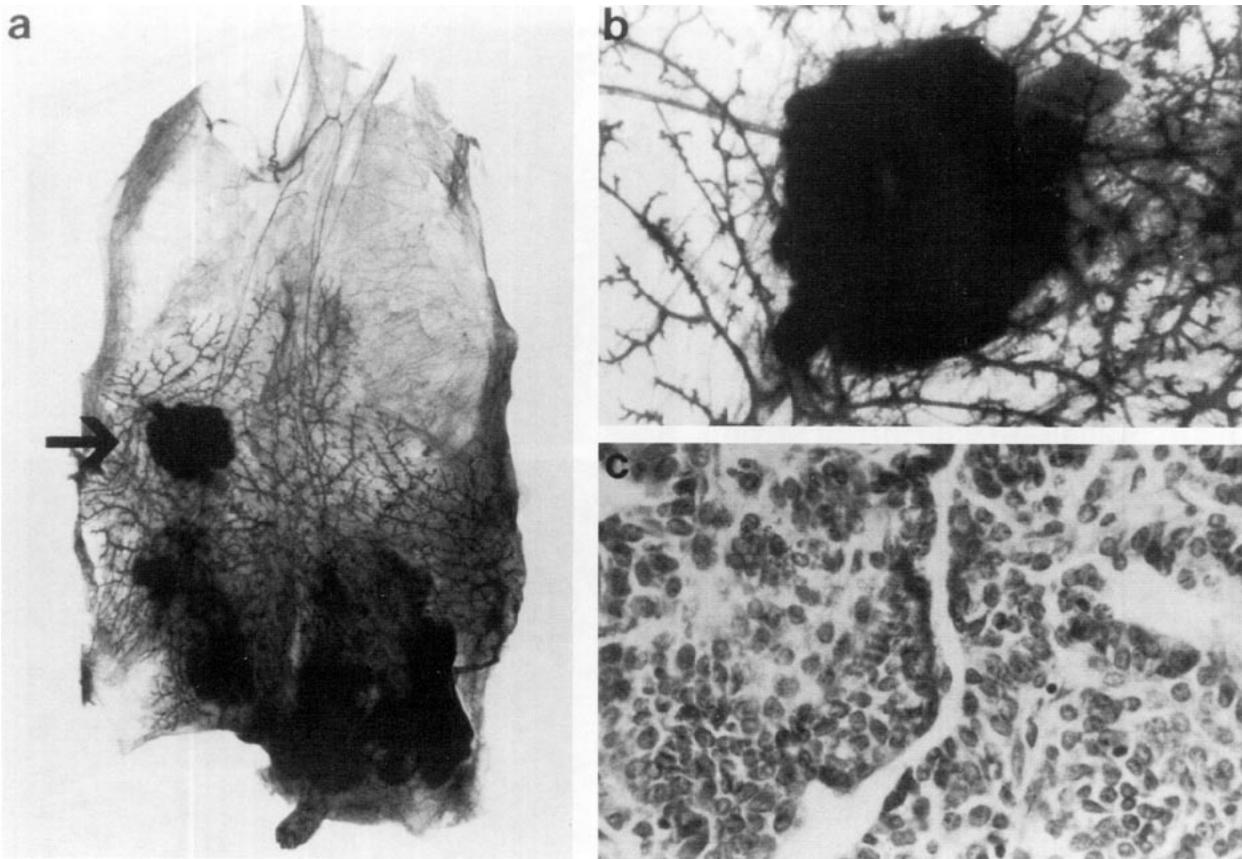


FIGURE 6. Histopathology of DMBA-induced mammary alveolar hyperplasia. (a) Whole mount of fat pad containing a focal lesion indicated by an arrow; (b) 5 \times magnification of this focal lesion; and (c) histopathological section of this lesion. From Zhang et al. (9).

not significantly differ in the frequency of carcinomas formation, while both frequencies were much greater than that found in the Cop mammary ectopic glands (Fig. 5). These results indicate that the presence of Cop cells did not modulate the susceptibility of neighboring WF cells to DMBA-induced carcinogenesis. Thus, the *MCS* gene product does not act intercellularly.

We have also begun to ask at which stage in carcinogenesis do the susceptibility and *MCS* genes act. Thus far, we have examined the very early stages of DMBA carcinogenesis: carcinogen activation and DNA adduction. Both these processes were studied in primary cultures of mammary epithelial cells to avoid any confounding systemic effects, since we knew that both classes of genes are mainly active within the mammary parenchyma.

Radiolabeled DMBA was added to WF, F344, and Cop rat mammary cell cultures. DMBA metabolites that were released into the medium were collected, isolated, and analyzed by reverse-phase HPLC. No qualitative or quantitative differences were found in DMBA metabolism in mammary cultures of these three strains (14). Next, DNA was isolated from DMBA-treated cells and assayed for DMBA-DNA adducts. Again, no qualitative or quantitative differences between cultures

from these three strains of rats were found (14). This suggests that it is likely that both the susceptibility and *MCS* genes act at carcinogenesis stages beyond DNA adduction.

Conclusions and Future Directions

In summary, we have found that various inbred rat strains differ in their susceptibilities to spontaneous and induced mammary carcinomas. The rate of spontaneous mammary carcinomas can be increased by genes acting within the pituitary that cause hyperplasia and neoplasia of the prolactin-producing cells. In contrast, spontaneous mammary carcinogenesis can be totally inhibited by the *MCS* gene, which acts mainly within the mammary epithelium. This gene also inhibits radiation, chemical, and hormone-induced mammary carcinomas. In rats lacking the *MCS* gene, the frequency of induced mammary carcinoma can be increased by dominant susceptibility genes acting within the mammary parenchyma. The susceptibility and *MCS* genes act at stages of carcinogenesis beyond carcinogen activation and DNA adduction.

The *MCS* gene is mammary carcinoma-specific in that it does not inhibit induced or spontaneous tumors in

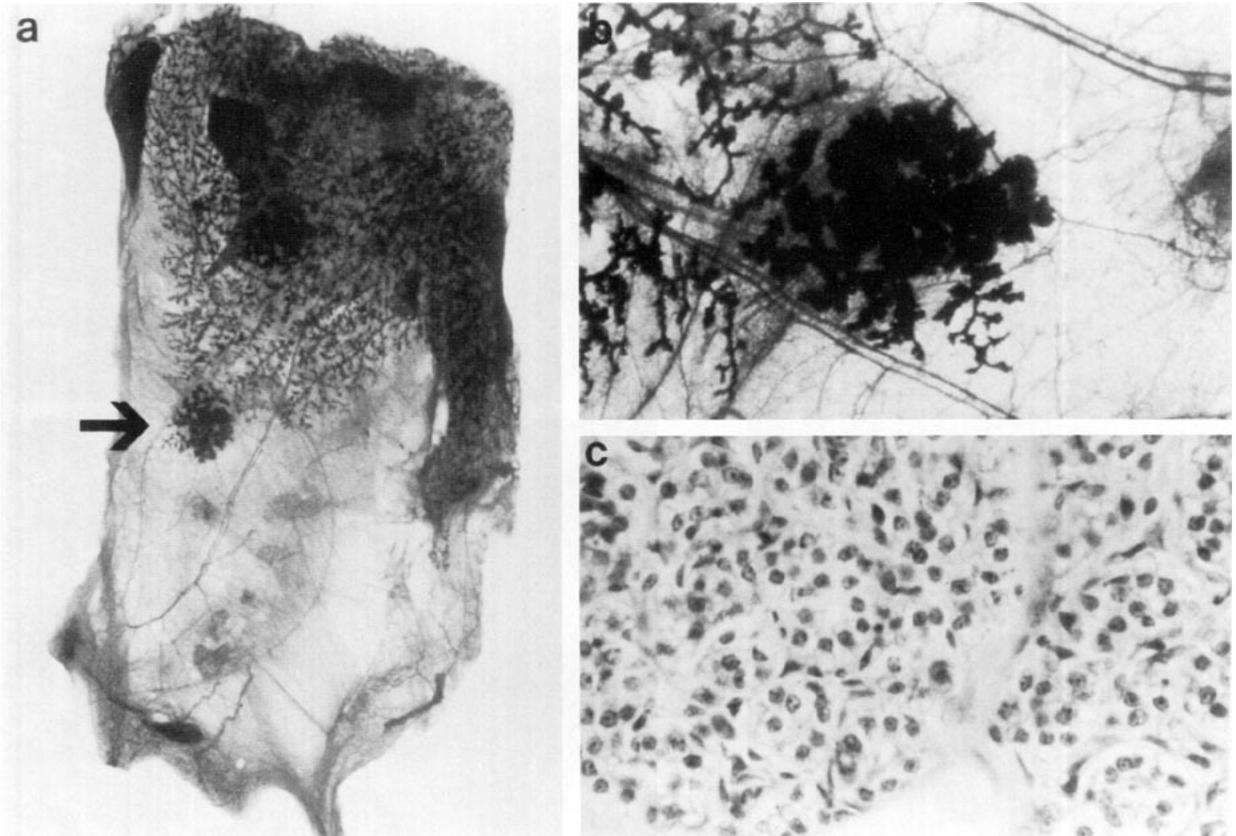


FIGURE 7. Histopathology of DMBA-induced mammary microcarcinoma. (a) Whole mount of fat pad containing a focal lesion indicated by an arrow; (b) 5 × magnification of this focal lesion; and (c) histopathological section of this lesion. From Zhang et al. (9).

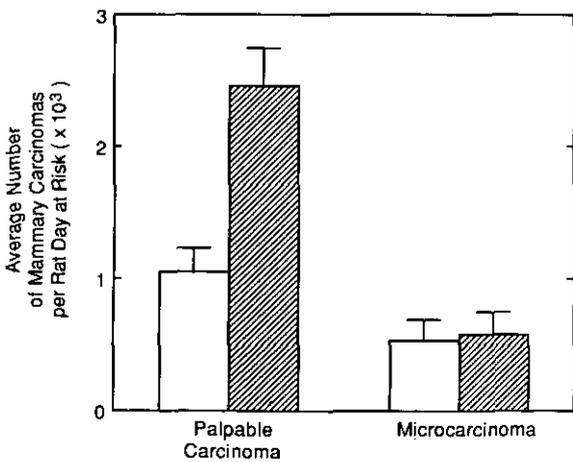


FIGURE 8. Average number of palpable and microcarcinomas developing at graft sites of WF mammary cells in different host strains. Bars represent mean values of each group ± SE. WF donor cells grafted into WF × Cop (□, n = 122); WF donor cells grafted into WF × F344 (▨, n = 99). From Zhang et al. (9).

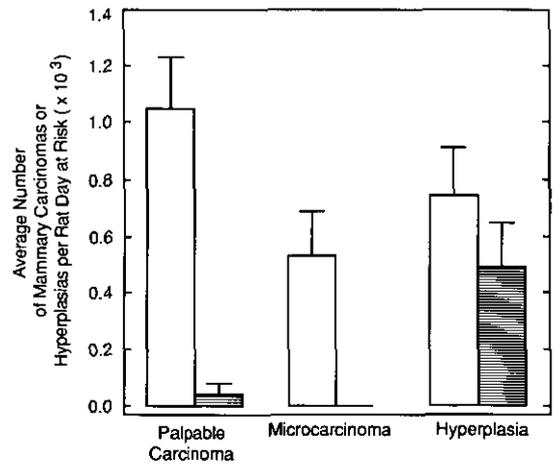


FIGURE 9. Comparison of average number of carcinomas or hyperplasia that developed at graft sites in WF × Cop F₁ rats. Bars represent mean values obtained from each group ± SE. WF donor cells grafted into WF × Cop (□, n = 122). Cop donor cells grafted into WF × Cop (▨, n = 90). From Zhang et al. (9).

other organs tested and also does not inhibit noncarcinoma carcinogen-induced pathologies within the mammary gland.

We are currently attempting to further characterize the function of both the susceptibility and *MCS* genes. For example, we and others have been unable to induce mammary carcinomas in Cop rats by a wide variety of physical, chemical, and hormonal carcinogens. We are currently attempting to overcome this *MCS*-induced resistance by introducing a series of activated oncogenes into the mammary epithelium of Cop and control strain rats. Defining oncogenes that can and cannot bypass the suppressor function of the *MCS* gene will aid us in understanding the mechanism of *MCS* action. Finally, we are also currently involved in devising strategies to molecularly clone and characterize the *MCS* gene. We are currently evaluating both subtraction hybridization and insertional mutagenesis as a means to achieve this goal.

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