THE MOUSE MAMMARY TUMOR SYSTEM

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My presence at this Symposium can be justified only by the fact that I have been asked to present a model tumor system which may be useful to you. Although multistage models for carcinogenesis have been proposed, most of these models lack well defined steps which can be enumerated and studied separately. The mouse mammary tumor system offers certain advantages since the various steps in the process of tumor formation can be handled separately.

In the mouse, mammary tumors arise from preexisting mammary gland hyperplasias [15], [16]. Similarly, it can be shown that these preneoplastic hyperplasias, in turn, arise from normal tissues [15], [16]. The hyperplastic areas can be identified and removed for study from living mammary gland tissues. Furthermore, they can be enumerated with great precision in stained mammary gland whole mount preparations. The preneoplastic hyperplasias include the classic hyperplastic alveolar nodules and other less well known lesions [2].

The preneoplastic nodules resemble the normal lobules seen in pregnant females [2]. They persist and can be identified in virgin or in nonpregnant, nonlactating parous females. They do not resemble neoplasms, however, according to histologic or cytologic criteria [21], hormonal requirements [3], [33], or metabolic behavior [28].

By means of the fat pad transplantation technique [15], it has been shown that mammary tumors arise in outgrowths derived from nodules, whereas nodules arise in outgrowths derived from normal tissues. These observations can be embodied in a simple schema (figure 1).

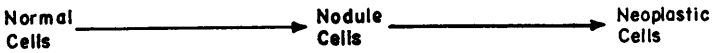

FIGURE 1

Simple schema for mammary tumors.

The three cell types included in the schema can be distinguished from one another according to a number of criteria. Some of these criteria are shown in table I. Intergrades between the normal cells and nodule cells, as well as between

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TABLE I

CHARACTERISTICS OF NORMAL, PRENEOPLASTIC, AND NEOPLASTIC MOUSE MAMMARY GLAND TISSUES

Characteristic	Tissue Type			Reference
	Normal	Preneoplastic Nodules	Neoplasms	
Deviation from normal morphology	none	little	great	[2]
Hormone dependence *in vivo* and *in vitro*	great	<normal	little	[3]
Overgrowth of normal ducts	rare	rare	usual	[19]
Fat pad dependence	usual	usual	rare	[15]
Insulin required for glucose utilization	usual	intermediate	rare	[28]
Outgrowth in gland free fat pad	normal	hyperplastic	tumor	[15]
Tumor producing capability	very low	low to high	—	[12]

nodule cells and neoplastic cells, are expected and some examples have been encountered. The three cell types, however, can be identified with remarkable accuracy and removed for study from living mammary gland tissue.

Mouse mammary noduligenesis and tumorigenesis occur following infection with appropriate viruses, the application of effective chemical carcinogens, or prolonged stimulation by certain hormones. In the rat, mammary noduligenesis and tumorigenesis follow the application of appropriate chemical carcinogens or X-ray irradiation. The role of these oncogenic factors in the nodule transformation and in the neoplastic transformation is being investigated. The role of viruses will be reviewed in some detail since these agents have been investigated most extensively.

The mouse mammary tumor virus (MTV) is transmitted from infected mothers to suckling offspring by means of milk [7], [8]. Occasionally it is transmitted from infected males to susceptible females at the time of copulation by means of the seminal fluids [9]. The MTV does not pass the placental barrier, hence mice can be freed from MTV by the removal of embryos immediately before birth and then foster nursing the young mice on MTV free foster mothers [1]. These susceptible MTV free mice can be reinfected by the injection or feeding of active virus preparations, especially during the first three weeks of life [1], [8], [34]. The hyperplastic alveolar nodules produced by MTV appear early in life and possess high tumor producing capabilities [35], [32]. Characteristic virus particles are revealed by the electron microscope in mammary tissues [20], milk [17], [27], [38], nodules, and in neoplasms [35], [36] from infected mice. The MTV gives a positive reaction in the nodule bioassay system [30], [31].

It is clear that the MTV interacts with normal mammary gland cells yielding nodule cells which in turn give rise to neoplastic cells. The schema presented above can be modified to reflect this fact (figure 2).

The one virus concept of mouse mammary tumorigenesis encountered difficulties when it was shown that the presence of characteristic virus particles in mammary tissue was not always associated with MTV activity as measured by the nodule assay test [35], [36]. Strain C3HfBALB/c mice have many nodules

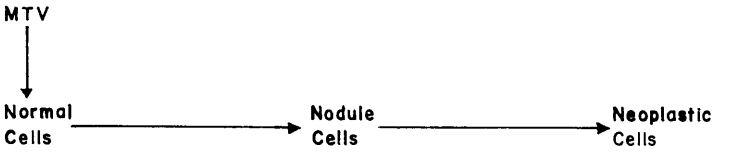

FIGURE 2

Modified schema showing interaction of MTV.

and a few mammary neoplasms late in life. (Embryos were removed from MTV infected C3H mothers and the young mice were foster nursed by MTV free BALB/c foster mothers.) These nodules and neoplasms contain abundant virus particles, as shown by the electron microscope [35], [36]. The virus carried by C3HfBALB/c mice only rarely is transmitted by milk [35] and day old BALB/c female mice only occasionally can be infected by the injection of virus preparations [34]. The C3HfBALB/c virus is regularly transmitted by either male or female parent to F_1 hybrids between C3HfBALB/c and BALB/c mice [35], [32], and the young are infected at birth. Although abundant nodules occur late in the lives of C3HfBALB/c females, these nodules possess very poor tumor-producing capabilities [11], [32]. Strains C3HfBALB/c or (C3HfBALB/c $\times$ BALB/c)F_1 mice yield negative reactions in the nodule assay test for MTV [30], [31]. In addition, rabbit antisera against C3HfBALB/c virus preparations will neutralize the MTV activity of virus preparations from C3H mammary gland tissues [10]. This observation suggests that common antigens are present in both viruses. There is reason to believe, however, that antigenic differences do exist [24]. The C3Hf virus is referred to as the nodule inducing virus (NIV) [32]. This name emphasizes the nodule inducing capabilities rather than the tumor producing capabilities of the C3Hf virus.

The occurrence in C3HfBALB/c mice of a second virus (NIV), capable of inducing both nodules and tumors, suggests that NIV can be substituted for MTV in our schema. Furthermore, there is reason to believe that both MTV and NIV coexist in C3H mice.

Another set of experiments shows that the nodule inducing capability of MTV can be blocked if the test mice are infected with NIV *in utero* and MTV is injected three weeks or more after birth. For example, NIV infected C3HfBALB/c mice three weeks or older when injected with MTV, failed to produce nodules, whereas nodules appeared in NIV free BALB/c mice when injected at any age with MTV. It appears that NIV is capable of interfering with the noduligenic action of MTV [34]. The Moloney mouse leukemia virus also interferes with the noduligenic capability of MTV when the former virus is injected into one day

old BALB/c females followed by the injection of the latter virus four weeks later [26].

These new facts suggest that mouse mammary noduligenesis and tumorigenesis can be due to the action and interaction of a number of related viruses. The possibility exists that other viruses and additional paths of interaction will be found. Although the schema may become complicated by the addition of several interacting viruses, the essential nature of the schema remains intact.

Mouse mammary tumors can be induced by means other than infection with mammary tumor viruses. Chemical carcinogens such as methylcholanthrene or dibenzanthracene, given by gastric intubation, will produce mammary tumors in both rats and mice [4], [5], [6], [18], [13], [23]. In BALB/c mice, mammary gland stimulation equivalent to pseudopregnancy or pregnancy is essential for nodule and tumor formation following treatment with chemical carcinogens [6]. In rats, mammary tumors can be produced by means of X-ray irradiation [37]. Finally, in certain strains of mice, and in the absence of demonstrable mammary tumor viruses or of chemical carcinogens, mammary tumors occur after prolonged hormonal stimulation [29], [22], [35]. Mammary hyperplasias resembling virus induced hyperplastic alveolar nodules occur in each of these mammary tumor systems. These hyperplasias increase in number with host age and are much more numerous in treated than in untreated animals of equal age [4], [5], [6], [18], [13], [23]. Final proof regarding the preneoplastic nature of the hyperplasias found in carcinogen treated mice is available [18], but in the case of carcinogen treated or X-ray irradiated rats, final proof must await the outcome of current experiments.

The proposed schema for mouse mammary tumorigenesis must be changed to include the interaction between normal mammary gland cells and several interacting viruses, chemical carcinogens, and prolonged hormone stimulation.

The nodule transformation can be induced by a variety of agents in both rats and mice, but the role of these agents in the neoplastic transformation has been investigated only recently. Nodule outgrowths derived from BALB/c females subjected to prolonged hormone stimulation in the absence of demonstrable mammary tumor viruses or of chemical carcinogens were studied. These nodule outgrowths were maintained in virus infected, or in chemical carcinogen treated isologous mice. To date, the neoplastic transformation has occurred in the nodule outgrowths subjected to infection with the MTV or exposed to chemical carcinogens [25]. At least these two agents are capable of inducing the neoplastic transformation in nodule cell populations induced by another agent. The nodule transformation and the neoplastic transformation, therefore, need not be induced by the same agent. The possible synergistic effects of the various tumor producing agents is being studied.

The simple schema for mouse mammary noduligenesis and tumorigenesis can be modified to include the known facts (figure 3).

In the rat, chemical carcinogens and X-ray irradiation result in the nodule transformation but their roles in the neoplastic transformation require additional study.

In conclusion, the mouse and rat mammary tumor systems offer many advantages to those interested in visualizing the process of tumor formation. The occurrence of recognizable preneoplastic nodules permits the separation of the tumor process into two clearly distinguishable stages. It is possible that the

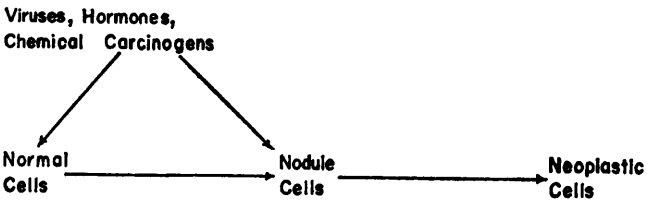

FIGURE 3

Modified schema summarizing mouse mammary noduligenesis and tumorigenesis.

nodule transformation represents only one of many sequential steps leading to tumor formation. In this case, its importance is due to the fact that it can be recognized in living mammary tissue and removed for subsequent study. In contrast, it is possible that the nodule transformation and the neoplastic transformation are the two *essential* sequential steps in the process of tumor formation. In support of the latter possibility is the occurrence in nodule cell populations of variant characteristics which do not appear to be directly related to the tumor producing capabilities of the nodules [12]. Similarly, the ability to override the local duct regulation [19], [14] appears to be a common characteristic of mammary neoplasms. Neoplasms differ among themselves, however, in many ways which do not appear to be related to this single common characteristic [14]. Regardless of this uncertainty, it seems clear that mouse mammary neoplasms rarely arise directly from normal mammary gland tissues.

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