Distribution of Developmental Markers in Rat Mammary Tumors Induced by N-Nitrosomethylurea

Renato Dulbecco, Barbara Armstrong, W. Ross Allen, and Marianne Bowman

The Salk Institute, The Monoclonal Antibody Laboratory of the Armand Hammer Cancer Center, La Jolla, California 92037

ABSTRACT

We have examined the distribution of immunological markers in 55 intraductal carcinomas induced in rats by N-nitrosomethylureas using several different regimens of carcinogen treatment. The goal was to determine the possible relationship of the marker distribution to those existing at various stages of development of the mammary gland. We have also examined two passage lines in syngeneic rats derived from two of the tumors. The distribution of markers was not affected by the regimen of administration. The primary tumors were found to maintain the general topography of mammary ducts but with infoldings of the basal layer without accompanying stroma. We attribute this to an abnormal expression of the tendency of basal cells to migrate towards the lumen to generate luminal cells. The distribution of markers in the tumors was evaluated by identifying 13 special features of distribution that are common in tumors, using all-or-none criteria. The distribution of these features bears out the high heterogeneity of tumors in which the various features vary in a seemingly independent way. There is also heterogeneity within tumors, adjacent nodes often having different marker distributions. The distribution of the markers is related to that found in the early stages of mammary development. Because of this characteristic and of the fact that the tumors contain both basal and luminal cells, they must originate from multipotent cells, probably the stem cells present in end buds and ducts as already proposed by other work. As they develop, the tumors can both differentiate towards the adult type and dedifferentiate towards a fetal type. Of the two transplanted lines one retained the same general features of primary tumors in several passages, whereas the other evolved into a fusiform cell type with a marker distribution not seen at any stage of mammary development. Foci of similar cells were already present in the primary tumor, suggesting that the tendency to progress was already determined at an early stage. The fusiform cells are similar and probably equivalent to the fusiform cells that arise in vitro in cultures of rat mammary cancers.

INTRODUCTION

The study of markers revealed by immunological methods in the cells of the rat mammary gland at different stages of development has permitted the identification of a number of different cell types and their developmental connections (1). This approach has permitted the tentative identification of the stem cells for mammary development mainly in terminal end buds but also in ducts. Transplantation of fragments of mouse mammary glands has furthermore suggested the existence of a second type of pluripotent cells devoted to alveolar development (2). This knowledge now allows an analysis of cell types and their developmental connections. The presence of different cell types, related to the normal types, has long been recognized in the cancers, using as distinguishing criteria the presence of keratin and collagen IV, transformation markers, ultrastructural features, casein production, hormone receptors, and hormone dependence. The origin of the tumors induced by either NMU or dimethylbenz(a)anthracene has been studied by determining the earliest lesions after carcinogen administration and by determining the fate of these lesions. The early lesions include hyperplasias of terminal end buds and formation of hyperplastic alveolar nodules or adenomas. The results suggest that the main alteration is in the end buds. The end bud role is also supported by the change of susceptibility of rats to cancer induction with age, depending on hormonal stimulation (14). In both cases the carcinogen is most effective when terminal end buds are most numerous and their cells most active in replication. Some ductal cells, however, also show a similar growth response.

The progression of the tumors was studied during several serial s.c. passages of tumors induced in inbred WF rats. An intriguing result is the strong expression of Thy-1 on the fusiform cells that appear in these tumors, in analogy to what happens in cultures of epithelial cells derived from rat mammary carcinomas. Cells probably similar to these have been observed in cultures from mouse mammary tumors. These cells have been interpreted to be of myoepithelial origin, but in fact they differ vastly from them in marker distribution.

MATERIALS AND METHODS

Carcinogenic Treatment. NMU was purchased from Sigma. Four groups of animals were used. The rat strains and modality of carcinogen administration used in each group are given in Table 1. Animals of two groups were primed for 9 days with a daily injection of estradiol (1 µg) and progesterone (1 mg) in order to promote duct and end bud development. The effects of these variables confirm results previously obtained by others. The main point is, however, that the variables do not affect the distribution of markers so that all the tumors can be considered together.

The questions that we try to answer in this work have been addressed already by others, using different criteria for defining cell types. The presence of different cell types, related to the normal types, has long been recognized in the cancers, using as distinguishing criteria the presence of keratin and collagen IV, transformation markers, ultrastructural features, casein production, hormone receptors, and hormone dependence. The origin of the tumors induced by either NMU or dimethylbenz(a)anthracene has been studied by determining the earliest lesions after carcinogen administration and by determining the fate of these lesions. The early lesions include hyperplasias of terminal end buds and formation of hyperplastic alveolar nodules or adenomas. The results suggest that the main alteration is in the end buds. The end bud role is also supported by the change of susceptibility of rats to cancer induction with age, depending on hormonal stimulation (14). In both cases the carcinogen is most effective when terminal end buds are most numerous and their cells most active in replication. Some ductal cells, however, also show a similar growth response.

The progression of the tumors was studied during several serial s.c. passages of tumors induced in inbred WF rats. An intriguing result is the strong expression of Thy-1 on the fusiform cells that appear in these tumors, in analogy to what happens in cultures of epithelial cells derived from rat mammary carcinomas. Cells probably similar to these have been observed in cultures from mouse mammary tumors. These cells have been interpreted to be of myoepithelial origin, but in fact they differ vastly from them in marker distribution.
All groups were terminated after 1 yr from the first injection. The rats were palpated at least once a month. After a tumor was felt, the animal was examined weekly. Tumors were collected when they were about 5 to 10 mm in diameter. When multiple tumors were present in the same animal, they were collected separately. Most tumors were subdivided into three parts. One was embedded in 2-chloroethane (Tissue-Tek II; Miles Laboratories, Naperville, IL) and immediately frozen at −50°C. Another was fixed in neutral buffered formalin and embedded in paraffin. The third part was used for DNA extraction and future studies of oncogenes and other changes.

Some of the tumors obtained in WF rats were transplanted s.c. into isogenic 3-wk-old female rats. The tumor fragment was minced and transplanted to one or two animals at different sites. Tumors developed in about 2 wk. Immunofluorescence studies were carried out in cryostat sections of frozen materials. The following monoclonal antibodies were used: 24B42 to luminal keratin (1, 21); 1A10 to basal keratin (1, 21); 9B16 to microvillini (1, 22); 57B29 to an antigen predominant on basal cells (1); 31G10 to Thy-1 (the Thy-1.1 allele) (23); and 48B45 to an antigen present on end bud cells and on some ductal luminal cells. This hybridoma was subsequently lost. The following polyclonal rabbit sera were also used: p-myo, to chicken gizzard myosin, a gift of Dr. J. Singer; p-ker, to muzzle keratin, a gift of Dr. J. Singer; and p-CIV, to collagen IV, a gift of Dr. L. Liouta.

Immunofluorescence was carried out by the sandwich method. In all cases a mouse monoclonal antibody and a rabbit polyclonal serum were used together in double immunofluorescence. p-CIV was used to locate the limits of the epithelial nodes. The slides were examined in a Zeiss fluorescence microscope with camera attachment. The characteristics of each tumor were recorded photographically, using a black and white ASA 400 film. Fifty to 100 photographs were obtained from each tumor, reflecting different markers, different areas of the tumor, and different sections.

Double immunofluorescence with two monoclonal antibodies of the same isotype was carried out by first staining with one, photographing selected fields, destaining, staining with the second antibody, and photographing the same field again (24).

RESULTS

Effects of Conditions of Carcinogen Administration on Tumor Yield. The data of Table 1 show that tumor yield was affected by some of the variables used, confirming observations reported by others. The most important variable was the amount of carcinogen administered (25, 26); the use of a high fat diet seemed also favorable at the lower dose used (2.5 mg of NMU per 100 g of body weight) (27). Efforts to determine whether the phase of the estrus cycle influences the tumor yield did not detect significant differences, probably owing to the low overall tumor yield, although the largest proportion of tumors was obtained when the carcinogen was injected at middle or late estrus (data not shown). None of the variables affected noticeably the distribution of markers and other properties of the tumors.

General Organization of the Tumors. Histologically, the tumors used in this study are papillary or cribriform ductal carcinomas (Fig. 1). They are formed of nodes containing cells with a moderate degree of pleomorphism, and at many places with a considerable number of mitoses. Hyperplasias and fibroadenomas were also observed, but they were not included in the study. Only two tumors gave evidence of infiltration and will be described in a separate publication.

The tumor nodules were surrounded by a BL identified by collagen IV and laminin, and with single or multiple lumina. The nodes were variably penetrated by strands of BL which, in serial sections, could be shown to be connected to the BL surrounding the node. The cell layer between BL and lumen varied in thickness, between one and many cells. Cells adjacent to the BL tended to have markers of basal cells (basal keratin, myosin, strong 57B29 antigen), whereas those away from it.
DEVELOPMENTAL MARKERS IN RAT MAMMARY TUMORS

Fig. 2. Double immunofluorescence. A and B, dissociation between BL and stroma in infoldings of the basal layer. BL is identified by p-CTIV (A), stroma by Thy-1 (B). Whereas the node is surrounded by a BL intimately associated with stroma, most of the infoldings do not contain stroma; only some of the larger ones do. Tumor D-38-p2, × 150. C and D, cells positive for Thy-1. C, Thy-1; D, collagen IV. Tumor D-7, × 625. E and F, cells with diffuse microvilli (end bud-like). E, microvillin; F, collagen IV. Tumor C11-1, × 250. G and H, cells positive for 57B29 antigen away from BL. G, 57B29 antigen; H, collagen IV. Tumor B-25, × 250.

tended to have luminal markers (luminal keratin, microvillin, weak 57B29 antigen). These basal cells, however, are unlike those present in adult ducts, which are strongly elongated, but like those present in the end buds: probably they are immature cells. The nodes appear therefore to original from end bud-like structures, and they often have a papillary architecture due to complex infolding of the basal cell layer.

Two observations are relevant for understanding how extensive infolding might have taken place. One is the absence of Thy-1-positive cells (characteristic of stroma) in association with BL infoldings within the nodes (Fig. 2, A and B). In contrast, the BL surrounding the nodes is tightly associated with such Thy-1.1-positive cells at the stroma side, as it is around normal ducts or end buds. The BL infolding in the tumors is not therefore produced by the pressure of the surrounding stroma. Also relevant are the occasional observations
Fig. 3. Double immunofluorescence. A and B, myosin-positive cells away from BL (arrow in B). A, basal keratin; B, myosin. Arrow in A indicates cell apparently moving away from the BL. Tumor C24, × 250. C and D, cells containing luminal keratin and myosin (arrows). C, luminal keratin; D, myosin. Tumor C6-p2, × 625. E and F, cells containing luminal keratin adjacent to BL. E, luminal keratin; F, collagen IV. Tumor D-7-3, × 250. G and H, cells containing both basal and luminal keratin. G, luminal keratin; H, basal keratin. The cells were stained first with antibody to luminal keratin, photographed, destained, and then stained with antibody to basal keratin. Tumor C-10-p2, × 250.

of cells with basal markers straddling the basal and luminal layer, suggesting that they are undergoing inwards displacement (see Fig. 4, A and B). A possible interpretation of these findings will be considered in the “Discussion.”

Distribution of Markers in the Tumors. The markers used and their localization with respect to the structure of the normal gland were described in our previous work on the characterization of cell types during the development of the normal rat mammary gland (1). In the tumors the localization or the grouping of markers is often abnormal. In order to analyze the many tumors and compare them to each other in an objective way, we identified several special localizations of these markers in the tumors, which deviate from their localizations in the glands of normal adult virgin 7-wk-old animals. Because these
special localizations do not exist in all tumors or in all parts of a given tumor, we classified all the tumors for presence or absence of each special localization; this makes it possible to obtain an overview of the localization of the markers in the tumors. The identification of the localization was carried out on photographs, in order to make it as objective as possible. A given tumor was listed as positive for the special localization of a given marker if that specialization was unambiguously observed in photographs taken at several different locations through the whole tumor. The classification of the tumors in respect to marker distribution is therefore of the all-or-none type. If in the following description of the special localizations, only one marker is mentioned, its behavior is considered separately from that of other markers.

We have identified the following special localizations. Arrangement 1, cells with total keratin, identified by p-ker, away from BL. This arrangement is present in end buds. Arrangement 2, epithelia positive for Thy-1 (Fig. 2, C and D), as is
 DEVELOPMENTAL MARKERS IN RAT MAMMARY TUMORS

The distribution of these features over the tumors is given in Fig. 5. From the data three conclusions can be drawn. (a) The tumors are very heterogeneous, many displaying different assortments of special localizations. Heterogeneity is found not only between tumors, but within tumors as well. Fig. 6, G and H, gives a typical example, showing two adjacent nodes: in one, the basal cells (identified by basal keratin) are myosin positive; in the other, they are myosin negative. (b) The distribution of the special localizations is not related to the conditions of tumor induction, such as dose of carcinogen, method of administration, or the rat strain. (c) The special localizations found in the tumors are also found in terminal end buds and partly in early ducts and end buds.

Effects of Serial Passages. Two tumor lines, B38 and C10, have been passaged several times s.c. in syngeneic rats. They behaved in different ways. B38 at the second passage generated a tumor line consisting of fusiform cells, whereas C10 continued to produce tumors similar to the primary tumor at all passages. The fusiform cells of the former line were loosely connected and lacked desmosomes, identified by either desmoplaques or keratoplaques (28). These cells entirely lacked myosin or basal keratin, contained luminal keratin, and were strongly positive for Thy-1, diffuse microvillin, 57B29 antigen, 45B31 antigen, and collagen IV (Fig. 6, A to F). In subsequent passages these characteristics persisted, although many cells became negative for luminal keratin and 45B31 antigen, two typical markers of epithelial mammary cells. The majority of cells continued to express 57B29 antigen, Thy-1, diffuse microvillin, and collagen IV. These cells were similar to the fusiform cells that arise in cultures derived from rat mammary tumors (15, 16). Some cells lacked all markers. A nodule of fusiform cells was observed at the first passage of the B-38 tumor.

DISCUSSION

Variables Affecting Tumor Production. The data reported in this paper were obtained from three different series of experiments, in which the regimen of carcinogen administration, the dose of carcinogen, the phase of the estrus cycle at the time of

Fig. 5. Distribution of the special marker arrangements over the tumors and normal structures. The arrangements are numbered 1 to 13, presence of the arrangement. The experiment and the tumor number are given on the right; when two numbers are used, the first denotes the animal, the second one the tumor. P, indicates passage with x the passage number. Special arrangements: 1, cells with polyclonal keratin away from BL; 2, Thy-1-positive epithelia; 3, diffuse microvillin; 4, 57B29-positive cells far from BL; 5, myosin-positive cells far from BL; 6, very few myosin-positive cells; 7, cells with luminal keratin and myosin; 8) cells with luminal keratin adjacent to BL; 9, cells containing both basal and luminal keratin; 10, basal cells without basal keratin; 11, cells with myosin but no basal keratin; 12, cells with basal keratin but no myosin; 13, cells with basal keratin away from BL. Squares with diagonal line, not done. Squares with dots, markers lost. A-D refer to groups in Table 1.

found in some early ducts and exceptionally in end buds. Arrangement 3, diffuse microvillin (Fig. 2, E and F), is found in end buds. Arrangement 4, cells positive for 57B29 antigen away from BL (Fig. 2, G and H); this situation is found in early ducts and end buds. Arrangement 5, myosin-positive cells away from the BL (Fig. 3, A and B); a similar situation occurs in end buds. Arrangement 6, myosin-positive cells almost absent (Fig. 4, E and F); this is a normal feature of prenatal ducts. Arrangement 7, cells with both luminal keratin and myosin (Fig. 3, C and D). Such cells are present also in normal glands (presumptive stem cells; Ref. 1). Tumors classified as negative had rare cells with this feature, with frequency comparable to that of 7-wk ducts. Arrangement 8, cells containing luminal keratin adjacent to BL (Fig. 2, G and H); this is also a regular feature of prenatal ducts and perhaps of end buds. Arrangement 9, cells containing both basal and luminal keratin (Fig. 3, G and H); this is a regular feature of early ducts and end buds. Arrangement 10, basal cells lacking basal keratin (Fig. 4, A and B); this feature is absent in normal glands. Arrangement 11, cells positive for myosin but negative for basal keratin (Fig. 4, C and D); similar cells may be occasionally present in end buds. Arrangement 12, presence of cells positive for basal keratin and negative for myosin (Fig. 4, E and F); such cells are present in early ducts. Arrangement 13, basal keratin in cells away from the BL (Fig. 4, G and H); this is a common finding in early ducts in the fetal or perinatal gland, and it occasionally occurs in end buds.
carcinogen administration, the rat strain, and the diet were different. A question is whether these different conditions affect the type of tumor generated, based on marker distribution. As shown in Fig. 4, this was not the case. We have considered therefore all tumors together in a single group. As an incidental observation we found that the conditions did affect the incidence of tumors, in accord with previous work. The amount of carcinogen administered appears to be the most important variable, as already reported (25, 26). We confirm the effectiveness of s.c. NMU administration (29). The lack of marked effect of the phase of the estrus cycle at the time of carcinogen administration is also in agreement with previous findings (30). This result suggests that the cell types in which the tumor initiates are not active in DNA replication exclusively at a specific cycle phase, because cells in DNA synthesis are most vulnerable (31). In the rat mammary gland the only structures...
showing DNA replication throughout the cycle are the terminal end buds (20). The end bud cells active at different cycle phases differ in some characteristics, but the differences may be the expression of different functional states. The point will be discussed further below.

Marker Distribution. In the tumors certain marker distributions tend to reflect the normal distribution in the adult gland: luminal cells are always positive for luminal keratin; basal cells, for basal keratin and strongly for 57B29 antigen. The basal cells, however, have immature morphology. Other distributions are abnormal. Thus of the luminal markers, microvillin may be diffuse at fissures between cells (as in end buds), instead of being strictly present at the lumen (as in ducts). Basal keratin and 57B29 antigen may be present in luminal cells, whereas luminal keratin may be present in basal cells. Basal cells may be myosin negative. The general epithelial marker 45B31 antigen is generally present on all cells, but in rare cases cells bordering on the lumen are negative for this marker. Thy-1.1 is expressed on epithelial cells in some primary tumors, although weakly and infrequently. These distributions are very different from those found in adult ducts, but they most closely resemble the distribution of the perinatal gland. Characteristics of distribution present in end buds are also more frequently represented in tumors.

From Which Cells Do the Cancers Originate? We will assume that the cancers are monoclonal in origin, although we have no direct evidence for it. In all cancers we observed multiple cell types. In all of them some cells have luminal markers; others have basal markers. The cancer must therefore arise from cells that are multipotent.

We have previously suggested (see Footnote 2) that there are two kinds of multipotent cells in mammary glands. One kind corresponds to the stem cells, located in end buds and in ducts, which can give rise to a complete mammary gland. The other kind corresponds to the alveoli-producing basal cells which are also present in ducts and are probably included among the basal cells. It seems that either cell type can give rise to both basal and luminal cells (1); therefore either type could be the origin of the cancers. End buds which contain stem cells have been previously implicated (9–12). It is unlikely that the cancers originate from the alveoli-producing cells of the ducts, because ductal cells replicate only at certain phases of the estrus cycle (20). The immature shape of the basal cells in the tumor nodes strengthens their similarity to end buds. It is likely, therefore, that the tumors originate in one of the immature cell types present in end buds. These structures are likely targets of the carcinogen because both show intensive cell multiplication in rats at the age at which they received injection (20); the replicative state of DNA makes them most vulnerable and prone to carcinogenesis (31).

The basic structure of the tumors is derived in all cases from a basic pattern corresponding to that of end buds. The tumors are made up of nodes, each surrounded by a BL associated with immature basal cells. Basal cells are at the periphery of the nodes, and luminal cells, internally. The organization within the nodes is, however, different from that of normal structures owing to penetration of BL strands and associated basal cells, sometimes forming a complex network. Most of the BL strands are not accompanied by stroma. This finding suggests that the penetration is accomplished by an ingrowth of BL and basal layer cells, possibly through a deviation of the normal development of basal cells. There is evidence suggesting that, in normal ducts, basal cells give rise to luminal cells by moving towards the lumen, but retaining, at least for some time, their connection to the BL (1). Under these conditions a massive migration of basal cells towards the lumen in a limited area would cause a BL introgression. We have indeed observed in some tumors pictures suggesting movement of basal cells towards the lumen (Fig. 4, A and B). This may be a frequent occurrence in the tumors; the movement may in some cases involve individual cells, causing the frequent appearance of individual myosin-positive cells away from the BL; in other cases, groups of cells may move together, causing infolding. Presumably stroma follows only the large infoldings.

Heterogeneity of Tumors. The tumors display marked heterogeneity in the expression of markers, both between individual tumors (Fig. 5) and within each tumor (Fig. 6, G and H). Heterogeneity affects every marker examined, usually independently of other markers. Part of the heterogeneity can be attributed to different degrees of cell differentiation; microvilli, for instance, may have either end bud or ductal distribution (32) at different places within the same tumor. Other types of heterogeneity cannot be attributed to this mechanism; for instance, absence of myosin expression in basal cells. The tumor cells evidently have mechanisms of variation in addition to normal differentiation.

Heterogeneity within tumors can occur at different architectural levels. Each node within a tumor is frequently homogeneous, but adjacent nodes may differ in marker distribution; heterogeneity is also found within nodes. The changes giving rise to heterogeneity appear therefore to occur randomly during the growth of the tumor, without consideration for its architecture, and to persist in the cell clone derived from the cell in which the change took place. Cells with different marker expressions are frequently in close proximity; it is therefore unlikely that the heterogeneity depends on local differences in concentration of factors needed for the expression of certain markers. Heterogeneity seems to depend therefore on random changes of gene expression affecting individual genes by an endogenous mechanism. The changes may be caused by either structural or regulatory alterations in the genes; whatever the mechanism, it must occur at a rather high frequency. The tumors, owing to their heterogeneity, are made up of many subclones. This observation does not, however, contradict the concept of tumor clonality, which relates to the origin of the tumor, not to its evolution.

Progression. Of two lines in which tumors were transplanted s.c. to isogeneic animals, one progressed, developing a cell type very different from any mammary cell type. These cells are fusiform; they retain expression of mammary epithelial markers such as luminal keratin, microvillin, and the surface antigens recognized by 57B29; they also express collagen IV, which is a basal cell marker, and Thy-1, which is occasionally present in small amounts on epithelial mammary cells (1). They do not express other basal cell markers such as basal keratin or myosin; many fail to express the 45B31 antigen which is a general epithelial marker. These cells were first recognized in a small node at the first passage and became prevalent from the second passage onwards. The formation of this cell type is probably not very frequent, because it did not appear in the other transplant line, nor was it seen in the numerous primary tumors examined. Its amplification during passage implies that it has a selective advantage under these conditions.

Differentiation and Dedifferentiation. In order to assess the nature of this cell variant, it is useful to consider first the general significance of the cell types present in the tumors. The marker distribution shows that they are related to cell types present at various stages of mammary development in early development of basal cells. There is evidence suggesting that, in normal ducts, basal cells give rise to luminal cells by moving towards the lumen, but retaining, at least for some time, their connection to the BL. Under these conditions a massive migration of basal cells towards the lumen in a limited area would cause a BL introgression. We have indeed observed in some tumors pictures suggesting movement of basal cells towards the lumen (Fig. 4, A and B). This may be a frequent occurrence in the tumors; the movement may in some cases involve individual cells, causing the frequent appearance of individual myosin-positive cells away from the BL; in other cases, groups of cells may move together, causing infolding. Presumably stroma follows only the large infoldings.

Heterogeneity of Tumors. The tumors display marked heterogeneity in the expression of markers, both between individual tumors (Fig. 5) and within each tumor (Fig. 6, G and H). Heterogeneity affects every marker examined, usually independently of other markers. Part of the heterogeneity can be attributed to different degrees of cell differentiation; microvilli, for instance, may have either end bud or ductal distribution (32) at different places within the same tumor. Other types of heterogeneity cannot be attributed to this mechanism; for instance, absence of myosin expression in basal cells. The tumor cells evidently have mechanisms of variation in addition to normal differentiation.

Heterogeneity within tumors can occur at different architectural levels. Each node within a tumor is frequently homogeneous, but adjacent nodes may differ in marker distribution; heterogeneity is also found within nodes. The changes giving rise to heterogeneity appear therefore to occur randomly during the growth of the tumor, without consideration for its architecture, and to persist in the cell clone derived from the cell in which the change took place. Cells with different marker expressions are frequently in close proximity; it is therefore unlikely that the heterogeneity depends on local differences in concentration of factors needed for the expression of certain markers. Heterogeneity seems to depend therefore on random changes of gene expression affecting individual genes by an endogenous mechanism. The changes may be caused by either structural or regulatory alterations in the genes; whatever the mechanism, it must occur at a rather high frequency. The tumors, owing to their heterogeneity, are made up of many subclones. This observation does not, however, contradict the concept of tumor clonality, which relates to the origin of the tumor, not to its evolution.

Progression. Of two lines in which tumors were transplanted s.c. to isogeneic animals, one progressed, developing a cell type very different from any mammary cell type. These cells are fusiform; they retain expression of mammary epithelial markers such as luminal keratin, microvillin, and the surface antigens recognized by 57B29; they also express collagen IV, which is a basal cell marker, and Thy-1, which is occasionally present in small amounts on epithelial mammary cells (1). They do not express other basal cell markers such as basal keratin or myosin; many fail to express the 45B31 antigen which is a general epithelial marker. These cells were first recognized in a small node at the first passage and became prevalent from the second passage onwards. The formation of this cell type is probably not very frequent, because it did not appear in the other transplant line, nor was it seen in the numerous primary tumors examined. Its amplification during passage implies that it has a selective advantage under these conditions.

Differentiation and Dedifferentiation. In order to assess the nature of this cell variant, it is useful to consider first the general significance of the cell types present in the tumors. The marker distribution shows that they are related to cell types present at various stages of mammary development in early
ducts or in end buds. The tumors do not have identical markers as the stem cells present in the adult gland, from which they appear to originate. The differences probably arise for two reasons: one is differentiation, as already discussed; the other is a process that goes in the opposite direction, that is, dedifferentiation. Dedifferentiation is shown for instance by the frequent presence in the tumors of cells containing both luminal and basal keratins but no myosin; such cells are characteristic of prenatal ducts. The question then arises of whether the fusiform cells of the passaged tumors can be also attributed to dedifferentiation. This possibility seems excluded because there is no corresponding cell during mammary development: none expresses strongly Thy-1 or microvillin; all express basal keratin and the 45B31 antigen, which are absent in most of the fusiform cells. So the variation of these cells goes beyond the normal developmental range. It is probably generated by events affecting the genome that are related to those generating heterogeneity.

The fusiform cells of the passaged tumor are quite similar to the fusiform cells that spontaneously appear during the growth in culture of epithelial cells derived from rat mammary tumors (15, 16). These cells are also tumorigenic when transplanted s.c. in isogenic host (16) and tend to replace the epithelial cells. It has been proposed that the cells appearing in vitro are of myoepithelial nature (18), but in fact the fusiform cells do not share with myoepithelial cells any of the markers except collagen IV.

The correlation between strong expression of Thy-1 and the high abnormality of this transplanted tumor may be significant. All the other markers investigated in this study have been shown to be present at some stage of mammary development. What is abnormal is not their presence in the tumors, but their distribution in respect to other markers and to the architecture of the epithelial structures. The perturbation of the distribution of differentiation markers is probably the sign of an altered program of gene expression insufficient to give rise to infiltrating cells. The passaged tumor expressing Thy-1 is the only one that would be considered anaplastic and highly aggressive. Thy-1 is expressed minimally and very transiently in normal development; the strong expression in this tumor may be considered ectopic. Ectopic expression of new gene functions, in addition to the perturbation of the normally expressed genes, may be required for the full expression of malignancy.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the gifts of specific sera and antibodies from Dr. J. Singer, Dr. L. Liotta, and Dr. E. Engvall, and the help of Dr. H. Battifora in the histological characterization of the tumors.

REFERENCES

Distribution of Developmental Markers in Rat Mammary Tumors Induced by \textit{N}-Nitrosomethylurea


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/10/5144

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/46/10/5144. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.