Histological Conformity of Implantation Tumors Produced by Kidney Cell Lines Derived from DimethylNitrosamine-treated Rats, with DimethylNitrosamine-induced Renal Mesenchymal Tumors¹

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ABSTRACT

The histology of five implantation tumors induced in rats by the deposition of cultured cell lines derived from dimethylnitrosamine (DMN)-treated rats is described and compared with the morphology of the predominant kidney neoplasm induced in vivo by a single high dose of DMN. The cell lines leading to growth upon implantation were long-established, continuously growing cultures obtained either from a DMN-induced renal mesenchymal tumor or from rats treated shortly before with a carcinogenic dose of DMN. The latter cultures had expressed morphological transformation at subcultures 5 or 6. All of the implantation tumors were of mesenchymal type, comprising variously a range of cell forms including fibroblast-like spindle cells, smooth muscle fibers, and “giant” cells, which resembled common aspects of the parent mesenchymal tumors induced in the rat kidney by DMN. Deposition of cells intrarenally illustrated the survival of remnants of preexisting nephrons as epithelial profiles scattered through the proliferating malignant tissue, a feature most characteristic of the parent tumor. The results confirmed the malignant nature of the various cell lines tested, in keeping with their altered behavior in vitro, and they were consistent also with the premise that the in vivo-in vitro system is selecting cells in culture that represent the same target population from which the renal mesenchymal tumors are derived in vivo.

INTRODUCTION

Over the past several years, efforts have been made in this unit to develop in vitro correlates of a potent model of nitrosamine-induced renal cancer for the study of the biology of chemical carcinogenesis. A single high dose of DMN¹ administered to protein-deprived rats is associated with a very high incidence, up to 100%, of renal neoplasia (6, 11). Histogenesis studies of the predominant neoplasm thus induced, a mesenchymal tumor, have indicated that advanced renal mesenchymal tumors have been established as continuously growing cell lines in vitro (3, 4). Secondly, in an in vivo-in vitro system, the isolation of kidney cells into primary culture from rats within 24 hr of a single injection of a carcinogenic dose of DMN has been shown to lead to the expression of morphological transformation, usually at the 5th subculture, as piled up colonies of densely crowded cells in random orientation (1, 5, 10). In contrast to kidney cells cultured from normal rat kidneys that have a finite life span, an average of 4 subcultures, the morphologically transformed cells derived from DMN-treated rats have persisted in culture for an unlimited period as continuously growing mesenchymal cell lines of a type similar to those derived from the mesenchymal tumors. Both transformed cell lines and tumor cell lines possess additional in vitro properties of neoplastic cells including enhanced plating and cloning efficiencies, colony formation in semisolid media, and agglutinability by concanavalin A (1, 3). The ultimate criterion of malignant character in vitro, however, is the production of implantation tumors in suitable test systems, and this paper records the general histological conformity between the implantation tumors produced by these renal cell lines and the parent tumor induced in vivo by a carcinogenic dose of DMN.

MATERIALS AND METHODS

Cell Lines. To date, 5 neoplasms have been produced in the course of continuing implantation experiments with lines of kidney cells established in vitro from rats receiving a carcinogenic dose of DMN. Two of these resulted from the transfer of a cell line obtained from a DMN-induced renal mesenchymal neoplasm, and 3 were from transformed cell lines derived from DMN-treated rats. The details of culture methodology have been described extensively in previous reports (4, 12). Cell line BMRI 21 was established in continuous culture as a population of mesenchymal cells derived from a 10-cm-diameter tumor growing in the kidney of a female Wistar albino Glaxo rat, 6 months after the injection of DMN (60 mg/kg body weight) administered at 6 weeks of age. Cell lines BMRI 38 and BMRI 40 were continuously growing, mesenchymal cell populations isolated from Porton albino Wistar rats at 24 and 2 hr, respectively, after the i.p. injection of DMN (60 mg/kg body weight). The dose of carcinogen was preceded by the feeding of a diet exclusively of sucrose and water for 3 days. Line BMRI 38 expressed morphological transformation at subculture 5 and BMRI 40 at subculture 6. Both cell lines exhibited properties consistent with neoplastic cells in vitro similar to those of cultured renal mesenchymal tumor cells (10, 12).

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³The abbreviations used are: DMN, dimethylnitrosamine; i.r., intrarenal.

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Implantation Procedures. The cell lines were adminis-
tered to host rats by a variety of procedures: by either s.c. or
i.p. injection into neonates; by s.c. injection in the flank of
6-week-old neonatally thymectomized (2) rats following
whole-body irradiation with 710 rads; or by i.r. deposition
of cells in 6-week-old rats following the administration of 7
p.o. doses at daily intervals of the immunosuppressant drug
niridazole (Imperial Chemical Industries, Sydney, New
South Wales, Australia). For i.r. implantation the cell pellet
was deposited subcapsularly at the posterior pole of the
right kidney with a blunted needle following exteriorization
of the organ through a dorsal flank incision under ether
anesthesia. In each procedure cells in log-phase growth
were stripped from culture flasks with trypsin-Versene,
washed 2 or 3 times with calcium- and magnesium-free
phosphate-buffered saline (0.8% NaCl-0.02% KCl-0.115%
Na₂HPO₄-0.02% KH₂PO₄), and suspended in Waymouth's Me-
dium 752/1 for implantation. Cell doses ranging from 10²
to 10⁶ cells were administered in a volume of 0.2 ml. Cells of
tumor growths had resulted from the implantation at the site of inoculation. In
each case, the tumor growths had assumed the form of fibrosarcomas containing predomi-
nantly spindle cells arranged loosely with an absence of
intercellular organization (Fig. 4). Several of the tumors,
normally appearing to smooth muscle fibers was also a feature, and
were the result of a similar process of sequestra-
tion of cords of hepatic parenchyma.

RESULTS

The tumors were found as large macroscopic growths 6
months after implantation at the site of inoculation. In
each case, the tumor growths had resulted from the im-
plantation of cells at a subculture stage later than passage 20.
In the rat in which transformed cells were deposited i.r.,
massive spread of the neoplasm occurred from the kidney to
the peritoneal cavity, body wall, and lungs substantiated their derivation
from the primary deposit from the normal structures by a
process of engulfment. In the invaded portion of liver, small
groups of epithelial cells were also visible among the tumor
cells for a short distance from the intact liver tissue (Fig. 8),
and these were the result of a similar process of sequestra-
tion of cords of hepatic parenchyma.

Histology. Samples of tumor tissue were fixed by immers-
ion in buffered formalin, and sections were stained with
Harris' hematoxylin and eosin, hematoxylin-phloxine-safran,
Van Gieson's collagen stain, Masson's trichrome,
Mallory's phosphotungstic acid-hematoxylin, and Gomez's
reticulin stain. The neoplasms contained liberal amounts of collagen
often present as thick tufts between cells. A well-developed
intercellular network of reticulin was also a prominent
feature (Fig. 6). The vascular supply was copious and, in
certain areas, the tumor tissue was dissected by intercon-
necting sinus-like channels.

Electron Microscopy. Samples of 3 tumors were exam-
ined by transmission electron microscopy. Diced 2-mm
pieces of tumor tissue were prepared according to a
conventional procedure by fixation in 2% OsO₄ buffered with
0.1 M sodium cacodylate and embedding in Epon. The
samples were stained with 2% uranyl acetate during thedehydration process and with Reynold's lead citrate on the
grid.

DISCUSSION

The predominant neoplasm induced in vivo by the admin-
istration of DMN (60 mg/kg) to immature rats is a mesen-
chymal tumor that displays a heterogeneous range of con-
nective tissue elements (6, 9). The basic component is a
fibroblast-like spindle cell infiltrating and proliferating in
the interstitial spaces between tubules and glomeruli of the
parenchyma. The organization of spindle cell ranges from
dense fibrosarcoma-like sheets to condensed nests of cells
characteristically distributed in concentric layers around
preexisting tubules. In other areas, the cell form is stellate
and the arrangement is reticular, closely resembling the
pattern of embryonic mesenchyme. Poorly cellular myoid
zones are also found. Smooth muscle fibers are invariably
present, either in sparse tracts or in profuse sheets assum-
ing the form of leiomyosarcoma. Rhabdomyoblasts seen as
microfibrillar giant cells are sometimes a prominent feature
of certain areas, while mature striated muscle, cartilage, or
osteoid is less frequent. The tumors are characterized by a
heavy deposition of collagen and reticulin, and abnormal
vascular tissue resembling hemangioendothelioma or he-
mangiopericytoma is not uncommon. Tumor cells with the
ultrastructural features of pericytes may be present. Scat-
tered throughout most parts of the tumor tissue are epithe-
lial profiles in the form of tuubes, cystic spaces, and solid
islands of transitional epithelium. In the past the presence
of these and the propensity for neoplastic spindle cells to
condense around some of the engulfed tubules has led to
confusion of this tumor entity with nephroblastoma. How-
ever, detailed light and electron microscopic scrutiny of the
advanced DMN-induced neoplasms and their early devel-
opment stages (6–9) leaves little doubt concerning the origin of such epithelial elements from preexisting tissue displaying collapse, dilation into cystic structures, or hyperplasia as pathological effects imposed by the infiltrating and expanding tumor tissue.

The histology of implantation tumors grown from DMN-induced rat kidney cell lines is of more than academic interest. In the first place neoplasms induced by cell lines derived from renal mesenchymal tumors should reflect some of the heterogeneous range of connective tissue cell forms characteristic of the parent tumors described above, but none of the epithelial profiles contained therein. Secondly, transformed cells derived from the kidney isolates of DMN-treated rats are believed to represent the same cell population as those propagated from the in vivo mesenchymal tumors (5), and they are therefore presumed to have common ancestry in a resident interstitial cell of the cortex (7, 8). If this premise is correct, then the histological spectrum of implantation tumors induced by transformed cell lines should match those produced by the cultured tumor cells themselves and should also reflect aspects of the parent DMN-induced renal tumors.

The histology in fact supports these contentions inasmuch as the implantation tumors displayed collectively a range of connective tissue forms including fibroblast-like spindle cells, smooth muscle-fibers, giant cells, collagen, and reticulin, thus resembling histological detail of the DMN-induced renal mesenchymal tumor. The highly vascular nature of the implantation tumors is further in keeping with the in vivo neoplasms, but common features of the parent tumors that were not represented in the 5 implantation tumors were embryonic mesenchyme, striated muscle, frank vascular neoplasm, or, in the renal implantation, characteristic concentric arrangement of cells around surviving renal tubule profiles. The lack of epithelial components in the extrarenal implantation tumors emphasizes the nonneoplastic nature of tubule profiles in the parent tumor, while the implantation tumor arising from i.r. deposit provides a useful model serving to illustrate the persistence of epithelial components sequestered from the preexisting renal parenchyma by the proliferating mass of malignant cells.

Although it was to be anticipated that cell lines derived from DMN-induced renal mesenchymal tumors would themselves give rise to similar mesenchymal tumors on implantation, it does confirm that the phenotypic nature of the cells has not been altered by prolonged maintenance in the artificial conditions of cell culture, despite a tendency for the cultures to assume an epithelioid appearance under the high population density of postconfluence (4). Furthermore, certain areas of the implantation tumors in which the cells were loosely arranged without intercellular organization reflected the morphological character of the cell lines in vitro with forms ranging from polygonal fusiform and spindle cells to polymorphonuclear giant cells (4, 5).

Of greater significance, however, is the histological conformity of the implantation tumors produced by transformed cell lines, on the one hand with the in vivo DMN-induced tumors and on the other with implantation tumors induced by cell lines derived from DMN-induced renal mesenchymal tumors. These observations (a) indicate that the morphological transformation in kidney cells isolated from rats shortly after a carcinogenic dose of DMN is compatible with the development of malignant transformation and (b) imply that the transformed cell lines and the renal mesenchymal tumor cells take common origin from a target cell population of mesenchymal lineage. The histological conformity between these implantation tumors and the parent kidney neoplasm confirms that the in vivo-in vitro system developed for studying the biology of chemically induced renal carcinogenesis is propagating a target cell population relevant to the in vivo development of the mesenchymal tumor. Finally, with the production of an implantation tumor by a transformed renal cell line derived from a rat dosed only 2 hr previously with the carcinogen, the study emphasizes once more that DMN acts rapidly, within several hr, in altering and programming target cells in the kidney to express malignant behavior in vitro (10).

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**REFERENCES**

Fig. 1. Fibrosarcomatous tumor arising from s.c. implantation of line BMRI 21. H & E, × 125.
Fig. 2. Electron micrograph of mesenchymal cells including fibroblast-like forms and intercellular deposits of collagen in s.c. implantation tumor from line BMRI 40. × 5,000.
Fig. 3. Electron micrograph of myofilamentous cell with dilated cisternae of rough endoplasmic reticulum. Implantation tumor from line BMRI 38. × 32,000.
Fig. 4. Pleomorphic cells loosely arranged without intercellular organization in peritoneal metastasis from i.r. implantation tumor. Line BMRI 38. H & E, × 500.
Fig. 5. Polymorphonuclear giant cell in implantation tumor arising from i.r. deposit of line BMRI 38. H & E, x 500.

Fig. 6. Well-developed intercellular network of reticulin in peritoneal metastasis. Implantation tumor from line BMRI 38. Gomori's reticulin stain. x 160.

Fig. 7. Implantation tumor growing in kidney shows glomerulus and tubules (arrows) sequestered within the mass of mesenchymal tumor cells. These epithelial profiles represent remnants of preexisting nephrons. Line BMRI 38. Hematoxylin-phloxine-saffron, x 160.

Fig. 8. Metastatic nodule in liver arising from i.r. implantation tumor. Epithelial profiles representing clumps of hepatic parenchymal cells are sequestered within the mass of mesenchymal tumor cells. Top, intact edge of normal liver. Line BMRI 38. H & E, x 125.
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