Characterization of an N-Methyl-N-nitrosourea-induced Autochthonous Rat Bladder Cancer Model

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ABSTRACT

Cohorts of 4- to 5-wk-old female Fischer 344 rats received four biweekly 1.5-mg doses of N-methyl-N-nitrosourea (MNU) intravesically and were sacrificed at various intervals. By 13 wk after initiation of the carcinogen, all animals have flat epithelial atypia and/or papillary transitional cell bladder carcinomas, and 67% of the lesions are histological Grade II or III. By 20 wk, 83% have gross bladder wall muscle-invasive tumors that eventually kill the host. There was no gross evidence of visceral metastases in any animal. This rat model of transitional cell carcinoma of the bladder is useful because: (a) all animals develop progressive neoplastic changes in situ within 4 mo after initiation of MNU treatment; (b) these lesions progress to grossly detectable bladder tumors which invade the bladder wall and kill the host; (c) this full progression of bladder epithelial cells from atypical hyperplasia through flat carcinoma in situ to transitional cell carcinoma occurs at discrete time points; (d) the histology of the grossly detectable tumors is that of invasive transitional cell carcinomas; and (e) no leukemias, breast cancers, lymphomas, or other non-bladder cancers are induced.

Six MNU-induced bladder wall-invasive tumors were karyotyped, and all tumors were diploid with 42 chromosomes. Three of the tumors had apparently normal karyotypes, while three tumors had karyotypes containing one or more cytogenetic structural markers. One of these markers (i.e., 8p+) was observed in two of the three tumors. The level of expression of total ras p21 (N-, Ki-, and Ha-ras p21) and codon 12-mutated c-Ha-ras p21 was determined by Western blot analysis. No increase in the total ras p21 nor any expression of codon 12-mutated c-Ha-ras p21 was detected in any of these tumors.

INTRODUCTION

Bladder cancer is the second most common urological malignancy in the United States with 46,000 new cases diagnosed and 15,000 deaths occurring each year (1, 2). In the past, investigators have studied the effects of therapy (3-8). These models, however, was the frequent development of squamous cell carcinomas of the bladder along with transitional cell carcinomas of the bladder. This same problem of induction of both squamous and transitional cell bladder cancers also occurs with the p.o. administration of BBN and FANFT (10-16). In this study, the methods used by Hicks and Wakefield (9) were modified by using a different inbred strain of rat (i.e., Fischer 344) and providing the animals with continuous antibiotics in their drinking water during carcinogen exposure to minimize urinary infections. Using these modifications, only transitional cell (no squamous cell) carcinomas of the bladder were induced. Using this modified method, the histological changes induced within the bladder epithelium following MNU exposure were temporally characterized.

Previous investigators studying human bladder cancer have demonstrated karyotypic abnormalities in some human bladder cancers in the region of chromosome 11p, where the c-Ha-ras oncogene is located (17-19). The human T24/EJ bladder cancer cell line is mutated at codon 12 of the ras p21 oncogene (20-22); furthermore, MNU-induced mammary tumors in rodents contain a glycine to glutamic acid mutation at codon 12 of the H-ras p21 oncogene (23, 24). Therefore, a series of MNU-induced transitional cell bladder cancers were characterized with respect to karyotypic changes, level of ras p21 expression, and the appearance of codon 12 mutant H-ras p21.

MATERIALS AND METHODS

An initial dose-response study was performed with a cohort of 4- to 5-wk-old female inbred Fischer 344 and inbred Copenhagen rats (Harlan Sprague, Indianapolis, IN). This study was performed to determine if tumors could be induced and to assess the histology of these tumors. The animals were anesthetized i.p. with Nembutal. One g of MNU (Sigma, St. Louis, MO) was dissolved in 100 ml of normal saline. The animals received 0.15 ml (1.5 mg) of this solution via a 22-gauge Teflon angiocatheter (Travenol Laboratories, Chicago, IL) intravesically under a continuous flow hood within 45 min of preparation of MNU solution, every other wk for a total of 2, 3, or 4 doses. The animals remained anesthetized for approximately 2 h after catheterization. Routine precautions were followed for working with a carcinogen. All animals received medicated water (a combination of trimethoprim sulfamethoxazole, neomycin, and polymyxin B) formulated as described previously (25) and Purina rat chow ad libitum. Animals were maintained on a 12-h light-dark cycle. Following this initial study multiple cohorts of 4- to 5-wk-old female Fischer 344 rats were used for this subsequent study to determine the temporal progression of these tumors. The animals were treated with 0.15 ml (1.5 mg) of intravesical MNU (Sigma, St. Louis, MO) via a 22-gauge Teflon angiocatheter (Travenol Laboratories) every other wk (i.e., wk 0, 2, 4, 6) for a total of 4 doses as described above. The animals were sacrificed at various intervals after they received their last dose of MNU.

Tissue Preparation

The animals were sacrificed by CO2 gas overdose and necropsy was performed. The urinary bladder and urethra were excised in toto.

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liver and lungs were grossly inspected for metastases and, if suspicious, were fixed in formalin or Bouin's solution, respectively. The kidneys and ureters were dissected and inspected for the presence of pyonephrosis, stones, or upper tract tumors secondary to reflux. The bladder was inflated with 10% buffered formalin, and a ligature was placed around the urethra. The bladders were fixed for at least 24 h and embedded in paraffin. Sections were cut transversely through the midportion of the bladder, and nine 5-μm sections were taken from each half to adequately sample the entire bladder; these sections were stained with hematoxylin and eosin. Large bladders completely filled with tumor were sampled for histology to document invasion. Some of the tumor tissue was prepared for karyotype and tissue culture, and the rest was stored at —70°C for Western blot analysis.

Light Microscopy

All sections from each bladder specimen were reviewed under low power. The section that appeared to have the greatest amount of change from normal rat bladder was selected for histological grading. Typically the histological changes were relatively uniform, but some areas of the bladder were occasionally more affected than other areas. The sections were assessed (14, 15, 26–28), staged by the UICC and the American Joint Committee tumor, nodes, and metastases system for human bladder cancer, and categorized (29) into either: (a) hyperplasia, flat or papillary atypia/dysplasia (only); (b) superficial TCC, which includes Stages P0 (papillary exophytic tumors with fibrovascular cores and nuclear pleomorphism of the epithelial cells with no evidence of invasion), P1 (tumors confined to the mucosa with full mucosal thickness of marked atypia/dysplasia (i.e., carcinoma in situ)), and P2 (tumors that demonstrate evidence of lamina propria invasion); or (c) bladder wall muscle-invasive transitional cell carcinomas. Atypia/dysplasia was mild, moderate, or marked and based on the demonstration of nuclear pleomorphism of size and shape, prominence of nucleoli, increased cellularity, loss of orientation, presence of giant cells, and presence of mitoses.

Chromosomal Analysis

Single cell suspensions were made by mincing tumors with scissors in RPMI 1640 (Gibco, Bethesda, MD) medium containing 10% fetal calf serum (Gibco, Bethesda, MD) and Colcemid (0.02 μg/ml). After incubation at 37°C for 10 min the medium was replaced with 0.075 M KCl hypotonic solution containing Colcemid (0.02 μg/ml) which was prewarmed to 37°C. The suspension was incubated at 37°C for 25 min and fixed with methanol/acetic acid (3:1). Chromosomal slides were prepared by dropping the cell suspension onto clean slides in a humid box. Thirty to 50 metaphases were counted for each tumor. Chromosomes were banded using the trypsin-Giemsa technique (30) and arranged according to the rat standard karyotype recommended by the committee for a standardized karyotype of Rattus norvegicus (31). Five to ten G-banded metaphases were karyotyped for each tumor.

Western Blot Analysis

Reagents. Rabbit anti-mouse Protein A-Sepharose was prepared by suspending 3 g of Protein A-Sepharose (Pharmacia, Piscataway, NJ) in 30 ml of 1 mili HCl for 15 min at room temperature and then washed on a sintered glass filter with 600 ml of 1 mili HCl, followed by 150 ml of RIPA buffer. The Protein A-Sepharose was transferred to a tube containing 9 ml of RIPA buffer and 6 mg of rabbit anti-mouse IgG (Organon Teknika Cappel, West Chester, PA) and allowed to react for 2 h at 4°C on a rotator. After washing 5 times with RIPA buffer, the rabbit anti-mouse Protein A-Sepharose is resuspended in 9 ml of RIPA buffer and stored at 4°C.

Methods. Gross tumors (i.e., greater than 0.5 g) were minced and homogenized in lysis buffer (1% Triton X-100:0.15 M NaCl:5 mM MgCl2:1 mM phenylmethylsulfonyl fluoride:1 mM leupeptin:2 mM EDTA in 0.05 M Tris-HCl, pH 7.4) using a Polytron homogenizer. Protein homogenates from normal rat bladders or bladders that received 2 or 3 doses of MNU were obtained by scraping the epithelial surface of the bladder with a glass slide, adding lysis buffer, and homogenizing in a dounce homogenizer to prepare an epithelial protein solution. The homogenates were then centrifuged at 15,000 × g for 15 min, and the supernatant was collected. The supernatant of each sample was assayed for protein content by the Bio-Rad (Richmond, CA) protein assay. The samples were then immunocentrifuged and concentrated. Two-mg samples for reacting with Ras 11 and 20-mg protein samples for reacting with E184 were prepared. The protein samples were added to 100 μl of rabbit anti-mouse Protein A-Sepharose and allowed to react at 4°C on a rotator for at least 30 min.

The resin was pelleted by centrifugation, and the supernatant was collected. Add this supernatant to 20 μl of Ras 11, and this was incubated on a rotator at 4°C for 1 h. Following this, 100 μl of rabbit anti-mouse Protein A-Sepharose solution were added and incubated at 4°C for 1 h. The solution was then pelleted, and the p21 remained bound to the pellet. The pellet was washed 3 times with cold RIPA buffer and twice with cold phosphate-buffered saline (pH 7.4). To the washed pellet were added 50 μl of sample buffer (10% 2-mercaptoethanol:2% (w/v) sodium dodecyl sulfate:30% (v/v) glycerol:0.025% bromophenol blue with a few crystals of methyl green in 0.05 M Tris-HCl, pH 6.8). This was then incubated at 100°C for 4–5 min. The samples were then pelleted, and the p21 was then in the supernatant which is loaded onto the gel.

Procedures for Western Blotting

The samples were run on a 12.5% polyacrylamide gel 1.5 mm thick with a 5% stacking gel. The gels were run at constant current of 20 mA/gel until the sample front reaches the 12.5% gel and thereafter at 40 mA/gel until completion. The gels were then transferred to nitrocellulose membranes as described previously (32). The blot that con-

### Table 1 Histopathological findings in bladders of female Fischer 344 rats treated with intravesical MNU and sacrificed at Wk 20 after the initiation of the carcinogen

<table>
<thead>
<tr>
<th>No. of doses of MNU</th>
<th>No. of rats sacrificed</th>
<th>% of rats with hyperplasia, flat or papillary atypia/dysplasia (only)</th>
<th>% of rats with superficial TCC of the bladder</th>
<th>% of rats with muscle invasion of the bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>19</td>
<td>36</td>
<td>45.5</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>0</td>
<td>17</td>
<td>83</td>
</tr>
</tbody>
</table>

### Table 2 Histopathological findings in bladders of female Copenhagen and Fischer 344 rats treated with 4 doses of intravesical MNU and sacrificed at 20 to 22 wk after initiation of the carcinogen

<table>
<thead>
<tr>
<th>Inbred strain</th>
<th>Wk of sacrifice</th>
<th>% of rats with hyperplasia, flat or papillary atypia/dysplasia (only)</th>
<th>% of rats with superficial TCC of the bladder</th>
<th>% of rats with squamous cell carcinoma of the bladder</th>
<th>% of rats with mixed TCC/squamous cell carcinoma of the bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copenhagen (n = 18)</td>
<td>20–22</td>
<td>22</td>
<td>17</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Fischer 344 (n = 12)</td>
<td>20–22</td>
<td>0</td>
<td>17</td>
<td>83</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3 Histopathological findings in bladders of female Fischer 344 rats treated with 4 doses of MNU at various time intervals after the intravesical initiation of the carcinogen

<table>
<thead>
<tr>
<th>No. of wk initiation of MNU</th>
<th>No. of rats sacrificed</th>
<th>% of rats with hyperplasia, flat or papillary atypia/dysplasia (only)</th>
<th>% of rats with superficial TCC</th>
<th>% of rats with muscle-invasive TCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-10</td>
<td>10</td>
<td>70</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>12-13</td>
<td>22</td>
<td>53</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>14-16</td>
<td>21</td>
<td>47</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td>17-18</td>
<td>10</td>
<td>50</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>20-24</td>
<td>12</td>
<td>0</td>
<td>17</td>
<td>83</td>
</tr>
</tbody>
</table>

RESULTS

Histopathological Progression of MNU-treated Bladders. In initial studies a dose-response relationship between intravesical MNU and rat bladder transformation to malignancy was determined. Female Fischer 344 rats require at least 3 doses of MNU if they are to develop TCC in the bladder in high frequency by 20 wk after initiation of the carcinogen (Table 1). The fourth dose increases the frequency of TCC to 100% and decreases the amount of time required to develop invasive TCC of the bladder. The female inbred Fischer 344 and Copenhagen rats differed greatly in their biological response to 4 doses of intravesical MNU. No Fischer 344 animal developed squamous cell or mixed squamous cell/transitional cell carcinoma of the bladder, while 50% of the Copenhagen bladders developed either squamous cell carcinoma alone or in combination with transitional cell carcinoma (Table 2). In addition, none of the Fischer 344 rats compared with 61% of the Copenhagen rats had diffuse squamous metaplasia of the bladder; similarly, 16% of the Fischer 344 compared with 78% of the Copenhagen rat bladders studied demonstrated focal squamous metaplasia. Approximately 24% (62 of 258) of all animals which were entered into MNU dosing schedules did not survive all 4 doses. The causes of death were typically related to complete ulceration of the urinary bladder or urosepsis secondary to urethral stricture formation and urinary obstruction. All Fischer 344 animals treated with 4 doses of intravesical MNU developed progressive neoplastic changes in their bladders (Table 3). These lesions progressed from hyperplasia (Fig. 1) to atypia (Fig. 2), carcinoma in situ (Fig. 3), and papillary transitional cell carcinoma (superficial TCC) (Fig. 4) to large bulky muscle-invasive tumors (Fig. 5) that completely filled the bladder lumen, obstructed the ureters, and killed the animal. These bladder tumors grew to 1 to 2 g in size (the normal rat bladder...
AN MNU-INDUCED RAT BLADDER CANCER MODEL

Weighs approximately 50 mg. The frequency of urinary calculi in the Fischer 344 animals in this study was less than 5% and, when present, contributed to the premature death of the animals. The frequency of urinary calculi in the Copenhagen rat bladders was 38%. No animal demonstrated gross or microscopic visceral metastases at any time during this study.

Chromosomal Analysis. Six muscle-invasive TCC bladder tumors induced in female Fischer 344 rats were karyotyped and studied for cytogenetic markers (Table 4). All 6 tumors had a diploid complement of 42 chromosomes. The karyotype of 3 of 6 (50%) tumors appeared normal with no cytogenetic markers, while 3 of 6 (50%) tumors had at least one cytogenetic structural marker and a numerical abnormality, i.e., monosomy 14, 8p*, and a marker chromosome in Tumor 1 (Fig. 6); tp(4;8), (q42;q24), and 8p* in Tumor 3 (Fig. 7); and 3p− in Tumor 5 (Fig. 8). In 2 of the tumors one of the cytogenetic markers is an 8p* structural abnormality.

ras p21 Expression. Using a mouse monoclonal antibody (Ras 11) which recognizes all 3 members of the ras oncogene family (N, Ki, and Ha-ras protein) whether normal or mutated, there were 0 of 17 MNU-induced bladder tumors analyzed that demonstrated a more than 2-fold increased expression of ras p21 as compared with normal bladder epithelium. Forty-eight % (i.e., 8 of 17) of the MNU-induced bladder cancers had a slight elevation of up to 2-fold in the expression of the ras p21 compared with the normal bladder epithelium, while the remaining 52% (i.e., 9 of 17) had less than or equal expression. As shown in Fig. 9, of a representative blot, Lane 1 is from normal whole rat bladders, Lanes 2 to 4 are from normal rat bladder urothelium-only preparations (excluding the bladder muscle) (Lane 4 is only a 1-mg preparation instead of 2 mg in the other lanes), Lanes 5 and 6 are urothelium-only preparations from animals that received 2 or 3 doses of MNU, respectively, and grossly did not have tumor, Lane 7 is normal whole rat bladder, and Lanes 8 to 14 are from MNU-induced tumors 1.5 to 2 g in size.

In more than 75% of MNU-induced rat mammary cancers, it has been demonstrated that the H-ras oncogene undergoes a codon 12 mutation which results in the substitution of glutamic acid instead of the normal glycine at position 12 (14, 23). Using the monoclonal antibody E184 which specifically recognizes this glycine to glutamic acid substitution at position 12 of the H-ras p21 in Western blotting, 4 of 5 rat mammary cancers induced by MNU, as described previously (33), were demonstrated to express the codon 12 mutant H-ras p21 in detectable levels. In contrast, multiple attempts to detect the expression of codon 12-mutated H-ras p21 in 15 different MNU-induced bladder cancers by Western blotting were unsuccessful.

DISCUSSION

Administration p.o. of various N-nitroso compounds can induce tumors in the rat urinary bladder. BBN when added to the drinking water or when administered by gastric gavage induces transitional cell and squamous cell carcinomas in almost all animals by 1 yr with 30 to 60% having invasive bladder cancers (13–16). However, BBN-induced bladder tumors have been reported to show marked quantitative and qualitative differences from human transitional cell carcinoma of the blad-
AN MNU-INDUCED RAT BLADDER CANCER MODEL

Fig. 5. Female Fischer 344 rat bladder treated with 4 doses of MNU with muscle-invasive TCC.

Table 4 Chromosomal analysis in six muscle-invasive bladder tumors induced by the intravesical administration of four doses of the carcinogen MNU

<table>
<thead>
<tr>
<th>Chromosome no.</th>
<th>Tumor Mode</th>
<th>% at the mode</th>
<th>Range</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>64</td>
<td>37–42</td>
<td>42,XX,-14,8p*, +mar (100%)*</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>80</td>
<td>37–42</td>
<td>42,XX (100%)</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>65</td>
<td>36–84</td>
<td>42,XX,rec(4;8)(q42;q24),8p* (75%)</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>82</td>
<td>39–42</td>
<td>42,XX (88%)</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>76</td>
<td>38–84</td>
<td>42,XX,3p* (100%)</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>87</td>
<td>39–42</td>
<td>42,XX (100%)</td>
</tr>
</tbody>
</table>

* Percentage of total metaphases karyotyped.

Fig. 6. Karyotype of Tumor 1 (42, XX, -14, 8p*, +mar). The arrow points to the structural abnormality, and M is the marker chromosome.

Fig. 7. Karyotype of Tumor 3 [42, XX, rec (4,8) (q42;q24), 8p*]. The arrows point to the structural abnormalities.

Fig. 8. Karyotype of tumor 5: 42, XX, 3p*. The arrow depicts the structural abnormality.

der (16). Administration p.o. of FANFT to mice produces urinary bladder tumors in approximately 70% by 11 mo, with tumors first appearing at 8 mo (3, 12–14). These tumors are transitional cell, squamous, and mostly mixed transitional cell and squamous cell carcinomas. Approximately 32% of these tumors are invasive by 11 mo and rarely metastatic to regional lymph nodes or lung (3, 12–14). One such FANFT-induced tumor has been established as a cell line and is termed MBT-2 (3–5, 11, 13, 14). MBT-2 is presently the most frequently used animal model of bladder cancer (3–8). This model has been studied extensively and is used to screen various systemic and intravesical chemotherapeutic agents. However, there are problems in using this model for the study of superficial TCC of the bladder. (a) It is an in vitro-passaged cell line that has been serially maintained in cell culture for over 10 yr. (b) It is usually transplanted s.c. [the reliability of implantation in the bladder is limited (34, 35) and, thus, one cannot assess treatment toxicity to the host urothelium] (c) It has a very fast in vivo growth rate [i.e., doubling time is less than 3 days (11)]. (d)
The tumor has lost its original transitional cell morphology, and by Day 34 after reimplantation into animals, it contains highly anaplastic sarcomatous elements (11, 13, 14). (e) The tumor is aneuploid with abnormal chromosomes, the development of which may have occurred during serial in vitro passage.

Based upon these limitations, a carcinogen-induced autochthonous transitional cell carcinoma bladder cancer model was developed based upon the original findings of Hicks and Wakefield (9, 36). Their original methods were modified by using a different inbred strain of rat (i.e., Fischer 344) and providing antibiotics in their water throughout the study. It was noted that, in Copenhagen rats given intravesical MNU, a high incidence of bladder calculi and squamous cell carcinomas was developed based upon the original findings of Hicks and Wakefield (9, 36). Their original methods were modified by using a different inbred strain of rat (i.e., Fischer 344) and providing antibiotics in their water throughout the study. It was noted that, in Copenhagen rats given intravesical MNU, a high incidence of bladder calculi and squamous cell carcinomas was induced. In contrast no squamous cell carcinomas and fewer calculi were induced by similar MNU treatment of Fischer 344 animals. Inbred strains of rats form spontaneous bladder calculi at varying rates (37). These differences in stone formation may have contributed to our findings. This MNU-induced model of bladder cancer has several advantages for the study of TCC for the following reasons. (a) It is a carcinogen-induced autochthonous bladder cancer model, not requiring metabolism by the gastrointestinal tract. (b) All animals develop progressive neoplastic changes in situ within 4 mo of initiation of carcinogen treatment. (c) These lesions progress to bladder wall muscle invasion and kill the host. (d) The histology of the invasive tumors which grossly develop is transitional cell. (e) The karyotypes are diploid with minimal chromosomal aberrations. (f) No leukemias, breast cancers, lymphomas, or other tumors are induced. (g) The full progression of bladder epithelial cells from atypical hyperplasia through flat carcinoma in situ to transitional cell carcinoma occurs at discrete times, thus, allowing this rat model to be used effectively in studying the molecular biology of bladder carcinogenesis. (h) The tumors are only of the urinary bladder epithelium.

Investigators studying human TCC bladder cancer have found considerable karyotypic heterogeneity with no consistent chromosomal abnormalities (17–19). Similar heterogeneity was seen in the MNU-induced tumors. Although all 6 MNU-induced muscle-invasive tumors that were karyotyped were diploid with 42 chromosomes, 3 of the 6 tumors had abnormal cytogenetic markers with 2 of the 3 having at least one consistent cytogenetic structural abnormality (8p+), and the third tumor having a (3p–) marker (Fig. 6). In the rat, c-Ha-ras-1 is localized to chromosome 1 (38); however, no cytogenetic markers on this chromosome were detected in the MNU-induced bladder cancers karyotyped.

MNU-induced rat mammary cancers contain a glycine to glutamic acid mutation at codon 12 of c-Ha-ras oncogene (23, 24). The human T24/EJ transitional cell bladder cancer cell line also contains a point mutation in codon 12 of the c-Ha-ras oncogene (20–22). In addition, c-Ha-ras p21 expression has been reported to be increased or activated in some but not all human bladder cancers analyzed directly from the patient (39–41) by Western, Southern, and immunohistocytocchemical analysis. However, recent studies shed some doubt on the importance of the ras oncogene in malignant transformation of human bladder epithelium (20, 21, 42, 43).

Using commercially available monoclonal antibodies Ras 11, which recognizes both normal and mutated N–, Ki-, Ha-ras p21, and E184, which recognizes the glycine to glutamic acid mutation at codon 12 of Ha-ras p21, it was determined that in MNU-induced bladder cancer there is no consistent overexpression of total ras p21 nor any detectable expression of the codon 12-mutated form of c-Ha-ras p21. Multiple blots were performed using 17 different MNU-induced tumors and multiple normal rat bladder samples. No apparent relationship between the level of ras p21 expression and malignant transformation was demonstrated. In fact, 52% of the tumors had similar or less expression of ras p21 than did normal rat epithelium when expressed on a per protein basis. In addition, after multiple attempts we were unable to demonstrate H-ras p21 mutations at codon 12 in any tumor specimens. Thus, the absence or low frequency of this mutation suggests that activation of the H-ras oncogene may not be necessary for malignant transformation of this in vivo rat model system. These results are in agreement with the previous demonstration that the in vitro treatment of rat urothelial cells leads to malignant transformation of these cells without inducing a mutation in the Ha-ras oncogene (44).

In conclusion, this rat model of MNU-induced bladder cancer appears to be ideally suited for further molecular genetic studies of carcinogen-induced transformation of bladder epithelium to transitional cell carcinoma of the bladder. In addition, since following MNU exposure, the bladder epithelium progresses through various early stages of neoplasia to gross muscle-invasive tumors at discrete time intervals and in high frequency. This model may be used in assessing the ability of various intravesical therapies to inhibit both the progression to invasive TCC and the growth of these tumors once they develop.4

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