ABSTRACT

The progression of prostatic intraepithelial neoplasia (PIN) to invasive prostate carcinoma has been analyzed in the C3(1)/TAG transgenic mouse model and appears very similar to the process proposed to occur in humans. PIN lesions in these transgenic mice histologically resemble those found in human PIN. Low-grade PIN was observed in the ventral and dorsolateral lobes at 2 months of age, whereas high-grade PIN was found in both lobes by 5 months of age. A progressive increase in the number of PIN lesions was observed with age. Prostate carcinomas, which appeared to arise from PIN lesions, were found by 7 months of age in the ventral lobe and 11 months of age in the dorsolateral lobe. Expression of TAg mRNA and protein in these lesions correlated with the development of PIN and carcinomas, as did the overexpression of p53 protein. Apoptosis levels were quite low in normal epithelial cells, moderate in low-grade PIN, and high in high-grade PIN and carcinomas. Levels of expression of proliferating cell nuclear antigen correlated with the degree of severity of the prostate lesions. Eighteen percent of PIN lesions were found to already harbor Ha-ras mutations, whereas 33% of carcinomas showed various mutations in Ha-ras, Ki-ras, and/or p53. Mutations in Ha-ras may, therefore, be an early event in a significant portion of PIN lesions. Because high-grade PIN showed many characteristics similar to those observed in carcinomas, we conclude that high-grade PIN is a precursor lesion of prostate carcinoma in this transgenic model. These transgenic mice will be useful to study mechanisms responsible for the progression of invasive carcinomas from PIN precursor lesions, as may occur during the development of prostate cancer in humans.

INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer among men throughout the Western world and has increased dramatically during the past decade (1). It is now the second leading cause of cancer deaths in American men and its incidence continues to rise. PIN is thought to be the precursor lesion of human prostate cancer (2). PIN develops in the peripheral zone of the prostate and shares a spatial association with prostate carcinoma. In addition, the microscopic features of PIN lesions share many morphological characteristics found in carcinomas (3).

Recently, considerable evidence has accumulated demonstrating multiple genetic changes in prostate carcinomas. Frequent allelic losses on chromosomes 7q, 8p, 10q, 16q, and 18q in prostate carcinomas have been found (4-8). In addition, aneuploidy in normal epithelial cells, moderate in low-grade PIN, and high in high-grade PIN and carcinomas, as did the overexpression of p53 protein. Apoptosis levels were quite low in normal epithelial cells, moderate in low-grade PIN, and high in high-grade PIN and carcinomas. Levels of expression of proliferating cell nuclear antigen correlated with the degree of severity of the prostate lesions. Eighteen percent of PIN lesions were found to already harbor Ha-ras mutations, whereas 33% of carcinomas showed various mutations in Ha-ras, Ki-ras, and/or p53. Mutations in Ha-ras may, therefore, be an early event in a significant portion of PIN lesions. Because high-grade PIN showed many characteristics similar to those observed in carcinomas, we conclude that high-grade PIN is a precursor lesion of prostate carcinoma in this transgenic model. These transgenic mice will be useful to study mechanisms responsible for the progression of invasive carcinomas from PIN precursor lesions, as may occur during the development of prostate cancer in humans.

MATERIALS AND METHODS

SV40 TAg Transgenic Mice. C3(1)/TAG transgenic mice have been described previously (13). Heterozygous TAg transgenic mice were maintained by breeding with FVB/N nontransgenic mice. Transgenic animals were identified by Southern and slot blot techniques using a 32P-labeled SV40-specific probe with mouse tail DNA (13). All manipulations of mice were performed in accordance with the guidelines of the Animal Care and Use Committee. Animal care was provided in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, 1985).

Histopathology. Male TAg transgenic and nontransgenic mice were sequentially sacrificed by CO2 asphyxiation from 2 months to 1 year of age. The male accessory sex glands were dissected and fixed in 4% paraformaldehyde. A horizontal slice of the ventral prostate, two horizontal sections of the dorsolateral prostate and three cross sections of each lobe of the seminal vesicles with anterior lobes (coagulating gland) were trimmed. Tissues were embedded in paraffin, and 4-μm sections were stained with H&E. Numbers of prostate lesions were counted individually using an ocular micrometer disc (Fisher Scientific, Pittsburgh, PA) under a light microscope, and the multiplicity of each prostate lesion was expressed as the average number per mm².

Immunohistochemistry. Sections were immersed in distilled water and heated by microwave for antigen retrieval, and the ABC method was performed (Vectastain ABC Elite kit; Vector Laboratories, Burlingame, CA). Anti-SV40 TAg mouse monoclonal Ab (PAb 101; PharMingen, San Diego, CA) and rabbit polyclonal antigens were used.
CA) was used at a dilution of 1:50; wild-type and mutant reactive anti-p53 sheep polyclonal Ab (Ab-7; Oncogene Science, San Diego, CA) was used at a dilution of 1:500; and anti-PCNA mouse monoclonal Ab (PC-10; Dako Corp., Carpinteria, CA) was used at a dilution of 1:400. To evaluate cell proliferation, the numbers of PCNA-immunoreactive cells staining positively in the nucleus (late G1, S and G2 phases) per 1000 cells were counted in normal epithelium and adenocarcinomas of the prostate. The number of cells counted in PIN were occasionally less than 1000 cells (400–800 cells) because of the relatively small size and multiplicity of the lesions. The percentage of PCNA-immunoreactive cells in the total number of cells was determined for each type of lesion.

**In Situ Hybridization.** SV40 virus was linearized with BamHI and inserted into the BamHI site of pGem7 (Promega Corp., Madison, WI). The SV40 early region was excited with SfiI and ClaI; the vector containing the SV40 early region was blunt-ended and religated to create pGTag. The plasmid was linearized with KpnI to generate a template for the sense probe or with ScaI to generate a template for the antisense probe or with ScaI to generate a template for the sense probe. (35S)CTP-labeled 2.4-kb sense and antisense RNA probes were generated by run-off transcription with Sp6 or T7 polymerase. In situ hybridization was performed as described previously (19). Briefly, histological sections were deparaffinized and hydrated, blocked in acetic anhydride and succinic anhydride, and hybridized overnight with riboprobes. Following RNase digestion and stringent washes, the slides were coated with Kodak NTB2 emulsion and incubated in the dark for 4 days. The slides were then developed in diluted D-19, fixed, and viewed with transmitted or darkfield illumination.

**In Situ End Labeling of Fragmented DNA.** Apoptotic cells in normal and prostate lesions were identified using the in situ end-labeling technique (ApopTag; Oncor, Gaithersburg, MD). Testes and thymus were used as positive controls. This technique not only identifies apoptotic bodies but also cells that have initiated apoptosis before they have formed apoptotic bodies. Cells with apoptotic bodies or positive staining with cell shrinkage were criteria for identifying apoptotic cells. The numbers of apoptotic cells per 1000 cells were counted in normal epithelium and adenocarcinomas; the numbers of cells counted in PINs were occasionally less than 1000 cells (400–800 cells) for the reasons described above. Apoptosis levels were expressed as a percentage of total cells. Areas of necrosis and regions immediately adjacent to necrotic areas within prostate carcinomas were excluded from the quantitation.

**Electron Microscopy.** Ventral prostates from mice at 4 and 9 months of age were dissected and immediately immersed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined under the electron microscope (model H7000; Hitachi, Tokyo, Japan).

**DNA Extraction from Paraffin Sections.** Multiple 5-μm paraffin sections (up to 10 sections) were individually placed on slides. The first and last sections were stained with H&E to confirm the presence of the lesions. Selected normal regions, PIN lesions, and adenocarcinomas were carefully scraped into a centrifuge tube with a disposable needle (avoiding necrotic areas), using the H&E-stained slides from the same block for orientation. The fragmented tissue samples were deparaffinized with xylene, centrifuged, washed in 100% ethanol, and air dried. The pellet was resuspended in a 1-mL solution containing 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5%Tween 20, and 10 mg/mL of proteinase K and incubated overnight at 55°C. DNA was extracted using phenol-chloroform, precipitated with ethanol and sodium acetate, and dissolved in TE buffer (10 mM Tris-1 mM EDTA).

**PCR-SSCP and Sequencing.** PCR-SSCP was performed for detection of Ha-ras, Ki-ras, and p53 mutations (20). Briefly, 50 ng of sample DNA were mixed in a total of 5 μL of reaction buffer containing 1 μL [32P]dCTP and amplified using a thermal controller (MT-100; MJ Research, Watertown, MA); 40 cycles at 94°C for 1 min, 55°C for 45 s, and 72°C for 1 min 30 s. PCR products were mixed with a solution of 95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol, heated at 94°C for 2 min, applied to 0.5% Mutation Detection Enhancement gels (AT Biochem, Malvern, PA) with or without 5% glycerol, electrophoresed for 9–13 h at 6 W at room temperature, and autoradiographed overnight at ~80°C. ras family genes were amplified by nested PCR. Primer sequences used are shown in Table 1. DNA from the CBT2 cell line was used as a positive control for a mutation in exon 5 of p53 (kindly provided by Dr. A. J. P. Klein-Szanto, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA; Ref. 21). Normal liver DNA from FVB/N control mice was used as a normal control. Mobility-shifted bands and representative normal bands in the SSCP analysis were cut out from gels and reamplified with the primers. Reamplified PCR products were purified and subcloned with the TA cloning kit (Invitrogen, San Diego, CA). The presence of the PCR product insertion in subclones using the pCR2.1 vector (Invitrogen) was confirmed by digestion with EcoRI, and inserts were sequenced (Taq DyeDeoxy Terminator Cycle sequencing kit; Applied Biosystems, Foster, CA) with a T7 promoter primer and a M13 reverse primer using a DNA sequencer (ABI Model 373A; Applied Biosystems).

### Results

**Histogenesis of PIN and Prostate Carcinoma.** Normal ventral and dorsolateral prostate were composed of a single layer of acini lined by columnar epithelial cells that contained pale cytoplasm (Fig. 1A). These epithelial cells were loosely arranged within sparse supporting connective tissue. The diagnostic criteria for human PIN was used for analyses of prostate lesions in the transgenic mice (3); low-grade PIN was composed of slightly increased cell numbers and cell density with normal amounts of cytoplasm or a sparse cytoplasm. Occasionally, focal piling up of cells was seen (Fig. 1B). Ultrastructurally, elongated nuclei but with relatively regular shape and size were observed (Fig. 2A). Unlike the human prostate, the mouse prostate has no continuous layer of basal cells. The retention of cellular polarity in the basal layer of cells was, therefore, used as a criterion for low-grade PIN lesions. In low-grade PIN, retention of the polarity of the basal layer of cells and intact basement membrane was confirmed by electron microscopy (Fig. 2A). At 5–6 months of age, lesions in transition from low- to high-grade PIN were seen frequently (Fig. 1C).

High-grade PIN was composed of a markedly increased cell population with stratification patterns identified as cribriform (Fig. 1D), micropapillary, tufting, or flat, as described previously in human high-grade PIN (3). The majority of cases had multiple patterns, but a flat pattern was rarely seen. Cellular atypia in high-grade PIN (Fig. 2B) were much more pronounced than in low-grade PIN (Fig. 2A). Nuclear chromatin was increased in density, and clumping was common. Prominence of the nucleoli with occasional multiple nucleoli was noted (Fig. 2C). Occasionally, epithelial cells had abundant cytoplasm and some disruption of the basal layer of cells was seen (defined by loss of cellular polarity in the basal layer; Fig. 2B), although an intact basement membrane was confirmed by electron microscopy (Fig. 2B and C). High-grade PIN in the transgenic mice
Fig. 1. Histogenesis of PIN and prostate carcinomas. A, normal ventral prostate epithelium; B, low-grade PIN; D and E, high-grade PIN; F-H, adenocarcinoma. Cellularity is slightly increased in low-grade PIN (B). Lesion in transition from low-grade to high-grade PIN with intact basal layer of cells (retention of the polarity of the basal layer) is observed at 5–6 months of age (C). Architectural patterns of high-grade PIN are cribriform (D) and papillary (E). The cell number in high-grade PIN is markedly increased, and cellular atypism is also seen. Strong cellular pleomorphism in adenocarcinomas is evident (F-H). G, arrows, adenocarcinoma arising from high-grade PIN with microinvasion into the acinar wall. Compression of surrounding tissue (G, arrowheads) and early invasion into the surrounding stroma are observed (G, arrows). Carcinoma cells are invading the adjacent stromal tissue (H). A-E, H&E, ×200; F and H, ×300; G, ×150.
was generally distinguished from low-grade PIN by the architectural pattern and cellularity. The categorization of lesions in transition from low-grade to high-grade PIN was based upon additional nuclear abnormalities, including alterations in nuclear shape and size, and chromatin density. As animals aged, multiple high-grade PIN lesions were observed (Fig. 1E). Apoptotic bodies were also seen frequently (Fig. 2, B and C).

In adenocarcinomas (Figs. 1, F-H, and 2D), cellular pleomorphism such as nuclear irregularity and varied size was much more pronounced than in high-grade PIN (Figs. 1, D and E, and 2B), and prominent nucleoli were often observed. Papillary, tufting, or cribiform patterns were often found, whereas the comedo pattern was rarely observed. Lesions exhibiting microinvasion were diagnosed as carcinoma (Fig. 1, F and G). Carcinoma was usually seen developing contiguously with high-grade PIN and showed microinvasion into the stroma (Fig. 1, F and G). Lesions in transition from high-grade PIN to carcinoma were also identified by a marked compression of the basement membrane and disruption of the basal layer of cells (Fig. 2D). Carcinoma cell invasion was limited to the surrounding area of stroma (Fig. 5C), and no metastases to other organs were seen in this series of animals studied. Apoptotic bodies were also frequently seen in carcinomas.

Chronological changes in the incidence and the multiplicity of PIN lesions and prostate carcinomas are illustrated in Fig. 3, A and B, respectively. In the ventral prostate, low-grade PIN was found in 83% (5 of 6) of mice at 2–3 months of age and in 100% (93 of 93) from 4 to 12 months of age. High-grade PIN was noted in both ventral and dorsolateral lobes by 5 months of age. High-grade PIN was seen in 88% (36 of 41) of mice from 5 to 8 months of age and 100% (46 of 46) of mice more than 8 months of age. A progressive increase in the number of PIN lesions per mouse was found with advancing age in both ventral and dorsolateral lobes. However, the ventral lobe was more frequently affected than was the dorsolateral lobe. Prostate adenocarcinomas were first observed at 7 months of age, where 19% of mice had carcinomas in the ventral lobe and 3% of mice had carcinomas in the dorsolateral lobe. After 8 months of age, prostate carcinomas were observed in the ventral lobe in 40% (18/45) of mice, whereas 13% (6/45) of mice developed carcinomas in the dorsolateral lobe. No histopathological changes were seen in the anterior prostate or seminal vesicles.

Expression of SV40 T<sub>Ag</sub> and p53. The results of T<sub>Ag</sub> and p53 protein expression during progression of prostate carcinomas are summarized in Table 2. Very few cells in the normal epithelium demonstrated positive nuclear staining for T<sub>Ag</sub> by immunohistochemical analysis. The number of immunoreactive epithelial cells increased in low-grade PIN (Fig. 4A) and was markedly elevated in high-grade PIN (Fig. 4B) and adenocarcinomas (Fig. 4C). This finding was consistent with the results obtained by in situ hybridization for T<sub>Ag</sub> mRNA (Fig. 5). The pattern of p53 protein expression (Fig. 4D) was similar to that for T<sub>Ag</sub> expression in normal tissues and in lesions of the prostate epithelial cells.

Apoptosis and Cell Proliferation in PIN and Carcinoma. Ultrastructurally, apoptotic cells contained fragmented nuclei and pyknotic chromatin (Fig. 2, B and C). The nuclear fragments occasionally contained a characteristic half-moon of condensed chromatin (top, right). Apoptotic changes were also frequently seen in carcinomas.
lesions of the ventral prostate are illustrated in Fig. 6A. Apoptosis levels in normal prostate epithelium were low at all ages (Fig. 4E). Low-grade PIN (Fig. 4F) consistently showed increases in apoptosis levels as compared to the levels of apoptosis in normal epithelium. In high-grade PIN (Fig. 4G), apoptosis levels were consistently high at all ages. Apoptosis levels of adenocarcinomas (Fig. 4H) were significantly higher than levels in normal prostate epithelium at 7–9 and 10–12 months of age. However, since some high-grade PIN lesions at 10–12 months of age had extremely low levels, apoptosis levels showed a large variation. Apoptosis levels of adenocarcinomas at 10–12 months of age were not increased over levels at 7–9 months of age. Average apoptosis levels in normal epithelium and prostate lesions from 2 to 12 months of age are presented in Table 3. Apoptosis levels were significantly higher in low-grade PIN, high-grade PIN, and adenocarcinomas compared to levels in normal epithelium. However, the apoptosis levels in high-grade PIN and adenocarcinomas were significantly higher than those in low-grade PIN.

Sequential changes in PCNA expression in different lesions of the ventral prostate are illustrated in Fig. 6B. The average levels of PCNA expression in each lesion are summarized in Table 3. The normal prostate showed a low rate of PCNA immunoreactivity at all ages. Levels of PCNA expression in all prostate lesions were significantly increased as compared to that of normal epithelium independent of age. PCNA expression correlated with the degree of severity of the prostate lesions. Expression was highest in adenocarcinomas and lowest in the low-grade PIN lesions, with mid-levels in high-grade PIN lesions independent of age.

**Mutations in PIN and Carcinomas.** The results of mutational analysis for Ha-ras, Ki-ras, and p53 in PIN and prostate carcinomas of the ventral lobe are presented in Table 4. Two of 11 (18%) PINs

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**Table 2 Expression of SV40 Tag and p53 proteins during progression of prostate carcinomas in C3(1)Tag transgenic mice as detected by immunohistochemistry**

<table>
<thead>
<tr>
<th>Protein expression</th>
<th>Normal-appearing epithelium&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Low-grade PIN&lt;sup&gt;a&lt;/sup&gt;</th>
<th>High-grade PIN&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adenocarcinoma&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VP</td>
<td>DLP</td>
<td>VP</td>
<td>DLP</td>
</tr>
<tr>
<td>SV40 Tag</td>
<td>±</td>
<td>–</td>
<td>1+</td>
<td>±</td>
</tr>
<tr>
<td>p53</td>
<td>±</td>
<td>–</td>
<td>2–1+</td>
<td>±</td>
</tr>
</tbody>
</table>

<sup>a</sup> Degree of immunoreactivity: –, negative; ±, rare; 1+, mild; 3+, moderate; 4+, strong. VP, ventral prostate; DLP, dorsolateral prostate.
Fig. 4. Nuclear expression of TAg protein (A–C), p53 (D), and apoptosis (E–H). Expression of TAg protein is seen in low-grade PIN epithelia (A, arrows) and at high levels in high-grade PIN (B) and prostate carcinoma (C). D, expression of p53 protein in prostate carcinoma. Invading carcinoma cells are also strongly immunoreactive (D, asterisk). A–C, ABC immunohistochemistry, ×300; D, ×150. Apoptosis levels are very low in normal epithelium of the prostate (E). Some apoptotic cells are seen in low-grade PIN (F, arrows), and many apoptotic bodies are found in high-grade PIN (G) and carcinoma (H). Note invading carcinoma cells with apoptotic bodies (H, asterisk). In situ end labeling of fragmented DNA, methyl green, ×300.
PIN AND PROSTATE CARCINOMA IN SV40 TAg TRANSGENIC MICE

Fig. 5. In situ hybridization of TAg mRNA in high-grade PIN (A, brightfield; B, darkfield, × 150) and prostate carcinoma (C, brightfield; D, darkfield, ×75). Note specific expression of TAg mRNA in high-grade PIN (B) and prostate carcinoma (D). mRNA signals are much stronger in high-grade PIN (A and B, arrows) than in low-grade PIN (A and B, asterisks). A strong signal is also seen in carcinoma cells invading the stroma (C and D, arrows).

(one case each of low-grade and high-grade PIN) had Ha-ras mutations in which there was a CAA to CTA transversion (Glu to Leu) at codon 61 (Figs. 7A and 8A). Four of 12 (33%) prostate carcinomas showed several gene mutations: two were Ha-ras mutations (a CAA to CTA transversion) at codon 61 (Figs. 7A and 8A), one was a Ha-ras mutation (an ATC to GTC transition; Ile to Val) at codon 84 (Figs. 7A and 8B), and one Ki-ras mutation, which was a GGT to GTT transversion (Gly to Val), at codon 12 (Figs. 7B and 8C). Since one carcinoma had a TCC to TCT mutation in codon 89 of the Ha-ras gene that did not change the translated amino acid, this result was excluded from the incidence of gene mutations in our analysis. A p53 mutation was found in only one case of prostate carcinoma in which an AGC to GGC transition (Ser to Gly) at codon 237 of exon 7 (Figs. 7C and 8D) occurred as well as an A to T mutation at codon 61 of Ha-ras. SSCP analyses of tail DNAs from mice demonstrating mutations in the prostate lesions did not show any abnormalities, confirming that the mutations were tumor specific.

DISCUSSION

In the C3(1)/TAg transgenic mouse model, prostate carcinomas appeared to progressively develop from PIN lesions in a manner very similar to the process suggested to occur in humans. We have determined in our transgenic model that high-grade PIN lesions share morphological and molecular biological characteristics with the prostate carcinomas. This model appears to provide a unique opportunity to investigate the spectrum of histological and molecular changes during the progression from PIN to invasive carcinomas.

The architectural patterns in human high-grade PIN reported by Bostwick et al. (22) were also observed in the transgenic mouse lesions, including tufting and micropapillary, cribriform, and flat patterns. Ultrastructurally, low-grade PIN showed an intact basal layer of cells and basement membrane, whereas high-grade PIN exhibited some disruption of the basal layer of cells as defined by changes in cell polarity, but the basement membrane remained intact. At later stages, high-grade PIN lesions appeared to progress into carcinomas with direct invasion through the acinar wall into the surrounding tissues with a disruption of the basement membrane.

SV40 TAg expression was detected at very low levels in normal epithelial cells of the prostate, but expression increased in low-grade PIN and was markedly increased in high-grade PIN lesions and carcinomas. The endogenous rat C3(1) gene is highly expressed in the rat ventral prostate with low levels of expression in the dorsolateral
Table 4 Mutations of Ha-ras, Ki-ras and p53 genes in PIN lesions and prostate carcinomas of C3(I)/TAG transgenic mice

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Age (mos.)</th>
<th>Histology</th>
<th>Gene</th>
<th>Exon/codon change</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>P18</td>
<td>4</td>
<td>L-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P19</td>
<td>4</td>
<td>L-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P20</td>
<td>4</td>
<td>L-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P21</td>
<td>4</td>
<td>L-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P24</td>
<td>6</td>
<td>L-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P23</td>
<td>7</td>
<td>L-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P15</td>
<td>7</td>
<td>H-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P25</td>
<td>7</td>
<td>H-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P26</td>
<td>7</td>
<td>H-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P16</td>
<td>10</td>
<td>H-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P17</td>
<td>11</td>
<td>H-PIN</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>CAA to CTAG</td>
</tr>
<tr>
<td>P5</td>
<td>7</td>
<td>Adenocarcinoma</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>ATC to GTC</td>
</tr>
<tr>
<td>P6</td>
<td>7</td>
<td>Adenocarcinoma</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>ATC to GTC</td>
</tr>
<tr>
<td>P11</td>
<td>7</td>
<td>Adenocarcinoma</td>
<td>Ha-ras</td>
<td>2/61</td>
<td>ATC to GTC</td>
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<tr>
<td>P2</td>
<td>8</td>
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<td>Ha-ras</td>
<td>2/61</td>
<td>ATC to GTC</td>
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<td>P22</td>
<td>8</td>
<td>Adenocarcinoma</td>
<td>Ha-ras</td>
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<td>ATC to GTC</td>
</tr>
<tr>
<td>P3</td>
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<td>Adenocarcinoma</td>
<td>Ki-ras</td>
<td>1/12</td>
<td>GGT to GTT</td>
</tr>
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<td>P7</td>
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<td>1/12</td>
<td>GGT to GTT</td>
</tr>
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<td>P10</td>
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<td>P4</td>
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<td>TCC to TCT</td>
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<td>P1</td>
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<td>Ha-ras</td>
<td>2/89</td>
<td>TCC to TCT</td>
</tr>
<tr>
<td>P8</td>
<td>12</td>
<td>Adenocarcinoma</td>
<td>Ha-ras</td>
<td>2/89</td>
<td>TCC to TCT</td>
</tr>
</tbody>
</table>

Table 3: Apoptosis and PCNA levels in normal epithelium, PIN, and prostate carcinomas in C3(I)/TAG transgenic mice from 2 to 12 months of age

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>No. of mice examined</th>
<th>Apoptosis levels (%)</th>
<th>PCNA positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>22</td>
<td>0.04 ± 0.07*</td>
<td>1.1 ± 1.6</td>
</tr>
<tr>
<td>Low-grade PIN</td>
<td>37</td>
<td>2.2 ± 1.7b</td>
<td>18.6 ± 12.3b</td>
</tr>
<tr>
<td>High-grade PIN</td>
<td>25</td>
<td>6.4 ± 3.7*c</td>
<td>41.5 ± 17.0c</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>7</td>
<td>5.7 ± 2.0d</td>
<td>65.5 ± 19.4d</td>
</tr>
</tbody>
</table>

a Mean ± SD.
b Significantly different from levels of normal epithelium; P < 0.01.
c Significantly different from levels of low-grade PIN lesions; P < 0.01.
d Significantly different from levels of high-grade PIN lesions; P < 0.01.

A similar pattern of T_{AG} expression is seen in the C3(1)/T_{AG} transgenic mice, with more frequent lesion development in the ventral lobe as compared to that in the dorsolateral lobe.

SV40 T_{AG} is thought to functionally inactivate the tumor suppressor genes p53 and Rb by directly binding these proteins (24, 25). Through its interaction with T_{AG}, the normal growth-suppressive functions of p53 are thought to be lost (25, 26). Earlier studies have shown that the interaction of the T_{AG} protein with wild-type p53 increases its half-life and steady-state level in cells (27, 28). The immunohistochemical detection of p53 protein consistently correlated with the immunoreactive patterns of T_{AG} expression. The accumulated p53 protein is, however, thought to be functionally inactivated (25, 26).

We were interested in determining at which stages during tumor progression changes in gene expression, cell proliferation, apoptosis, and mutations of relevant genes occurred. Tumor growth appears to be controlled by a balance between the rates of cell proliferation and cell death. Levels of cell proliferation as assessed by PCNA expression correlated to the degree of severity of the prostate lesions in the prostate (23). A similar pattern of T_{AG} expression is seen in the C3(1)/T_{AG} transgenic mice, with more frequent lesion development in the ventral lobe as compared to that in the dorsolateral lobe.

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transgenic mice and is consistent with observations in human prostate cancer (18, 29, 30). Reduced apoptosis is thought to be an important factor in accelerated tumor growth. A recent study of human prostate cancer has reported that high-grade PIN and carcinomas have increased rates of cell proliferation and apoptosis (18). It was also recently shown that a positive correlation exists between the apoptotic index and histological stage in human prostate cancers (31). We have found similar changes in apoptosis levels during prostate tumor progression in the T_{AG} transgenic mice. However, it has recently been reported that metastatic prostate cancer tissues have an increase in proliferation and a decrease in apoptotic cell death compared to localized prostate cancer (18). At late stages of prostate cancer progression, suppression of apoptotic control may lead to unrestricted cell growth and may be a critical event in the late stage of prostate tumor progression. Since no metastases were observed in this series of transgenic mice, we could not determine whether alterations in apoptosis levels occurred during metastatic progression.

We have recently reported that apoptosis levels are suppressed during the progression from preneoplastic lesions to carcinomas in mammary glands of female C3(1)/TAG transgenic mice through a p53-independent mechanism (26). Control of apoptosis during prostate tumor progression in the C3(1)/TAG male mice may similarly be p53 independent. However, in contrast to the mammary carcinomas, apoptosis levels in the prostate carcinomas were not reduced compared to the precursor PIN lesions. This may be one factor accounting for the relative slow growth of the prostate carcinomas in the T_{AG} transgenic males compared to the growth rate of mammary carcinomas of the T_{AG} transgenic females.

Specific point mutations in ras genes have been implicated in the development of a variety of human tumors (32). Overall, the frequency of ras gene mutations in prostate carcinomas is very low in U.S. men and, when present, generally are Ha-ras gene mutations (33–35). However, in Japanese men, Ki-ras mutations are found in approximately 25% of prostate carcinomas (36–38). This difference in frequency of ras mutations between U.S. and Japanese men may indicate different genetic susceptibilities by race or perhaps environmental influences. Carter et al. (33) suggested that ras gene mutations may play a role in the progression of prostate cancer in the U.S. men. Animal models have provided some evidence for a role of the ras oncogene in prostate tumor development. It was shown that the ras and myc oncogenes synergistically induce tumors when introduced into cells, forming a reconstituted mouse prostate (39). It was also reported that the transfection of the Ha-ras gene into Dunning rat prostate tumor cell lines increases the metastatic potential of these cells (40). Thus, although data from human studies has been inconclusive, animal experiments have provided evidence that the ras oncogene is capable of influencing prostate carcinogenesis and progression. We, therefore, wished to determine whether ras mutations might be involved in tumor progression in the C3(1)/T_{AG} transgenic mice.

ras mutations were found early during tumor progression in the C3(1)/T_{AG} transgenic mice. Eighteen % of PIN and 33% of carcinomas had ras mutations. The spectrum of ras gene mutations found in the transgenic prostate lesions have been reported in human prostate tumors, i.e., a CAA to CTA transversion at codon 61 in Ha-ras gene (33, 34) or a GGT to GTT transversion at codon 12 in Ki-ras gene (36). Although a mutation in codon 84 of Ha-ras was also observed, the biological significance of this mutation remains unclear since this alteration has not previously been identified as transforming. One transgenic tumor had mutations in both p53 and Ha-ras. Since the function of p53 in these transgenic mice is already impaired by binding to T_{AG}, the significance of the p53 mutation observed in this study is unclear. Mutations involving the p53 gene appear to occur at late stages of human prostate cancer development (41–43). The fact that some PIN lesions contain Ha-ras mutations suggests that ras mutations may be an early event in a significant portion of PIN lesions induced by T_{AG}.

This transgenic model will be useful to further study mechanisms responsible for the development of carcinomas from precursor PIN.
lesions, a transition that appears to be critical for the progression of prostate cancer in humans.

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