Morphological, Biological, and Biochemical Characteristics of Human Bladder Transitional Cell Carcinomas Grown in Tissue Culture and in Nude Mice


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ABSTRACT

The morphological, biological, biochemical, and karyotypic characteristics of four human bladder transitional cell carcinoma lines, SW-780, SW-800, SW-1738, and SW-1710, were investigated. In tissue culture, each cell line presented a distinct phenotypic expression. All but line SW-1710 grew when transplanted in the nude mouse. Light and electron microscopic studies showed morphological characteristics similar to the tumors of origin, being independent of the passages in tissue culture or the nude mouse. Lactate dehydrogenase was present in culture medium, tumor cell extracts, and the plasma of nude mouse-grown tumors, showing isoenzyme quantitative distribution typical for each cell line. In addition, each cell line exhibited a unique genetically determined enzyme phenotypic profile which, along with the karyotypic analysis, makes their identification feasible. These characteristics make the described tumor lines a valuable tool in studying various aspects of the biology of human bladder transitional cell carcinoma.

INTRODUCTION

The establishment of long-term tissue culture lines of various human tumors has enabled cancer investigators to undertake the study of certain biological characteristics of these cancers. Furthermore, the introduction of the nude mouse model and its ability to sustain a large number of human neoplasms following transplantation of established tumor cell lines or primary tumors removed at surgery have further expanded the scope and goals of experimental studies of human neoplasia. Thus, tumor growth characteristics, metastatic behavior, and response to single-agent treatment and combination chemotherapy have become feasible, promising a new era in our understanding of the multifaceted behavioral aspects of human cancers. Such studies, however, could be greatly facilitated by careful characterization of various human tumor lines available in vitro or in vivo prior to undertaking any in-depth study of their specific characteristics.

Human transitional cell carcinoma of the urinary bladder demonstrates great variability in morphological and biological behavior. Multifocal growth of the primary neoplastic process, invasion, metastases, and recurrence, even after a seemingly successful treatment at a very early stage, are characteristics of this type of neoplasia. In an effort to facilitate the study of the biology of this tumor, investigators have attempted to establish permanent tumor cell lines. Despite these efforts, however, only a very small number of transitional cell carcinoma cell lines have been successfully established, and of these, only few have been adequately studied (2, 3, 6, 17, 19).

This paper intends to present information on the behavioral characteristics of 4 human transitional cell carcinoma lines of the urinary bladder, SW-780, SW-800, SW-1738, and SW-1710, in both tissue culture and the nude mouse. Our approach has been to obtain information about their growth characteristics, morphological appearance, ultrastructure, production of marker macromolecules, such as lactate dehydrogenase, chromosome changes, and their enzyme phenotypic profile.

MATERIALS AND METHODS

Cell line SW-780 was established on July 31, 1974, from the bladder tumor of an 80-year-old Caucasian female following total cystectomy. Histopathologically, the tumor was characterized as Grade I transitional cell carcinoma (Fig. 1). Preoperatively, the patient had been treated with intravesical thiotaque without success. Tumor involvement of the vaginal wall was confirmed in March 1975, and a biopsy was read as Grade II transitional cell carcinoma. Metastatic lung disease was confirmed 2 months later. The patient received only supportive treatment and died on October 8, 1975.

Cell line SW-800 originated from a transitional cell carcinoma of the urinary bladder of a 54-year-old Caucasian male on August 16, 1974. The patient initially presented with hematuria on December 27, 1973. Urinary cytological evaluation at that time was reported as Class IV. On January 3, 1974, a transurethral tumor resection was carried out. The resected tumor was characterized as transitional cell carcinoma, Grade I. Tumor recurrence was noted a few months later, and a second transurethral tumor resection was performed on May 21, 1974.

This procedure was repeated on August 16, 1974. The resected tumor from which the present cell line originated was characterized as transitional cell carcinoma, Grade I, evolving into a Grade III cancer having, in places, the characteristics of a " spindle cell epithelioma." This was followed by radical cystectomy and pelvic radiation. The patient was seen for the last time on August 28, 1981. He was in good health and free of metastatic disease.

Cell line SW-1710 was established on August 8, 1977, from the bladder tumor of a 55-year-old Caucasian male. At the time of hospitalization, the patient complained of painless hematuria and urinary frequency for the last year. Cystoscopic examination revealed the presence of a multifocal papillary tumor occupying primarily the left lateral wall of the bladder and the bladder neck anteriorly. The tumor was resected through a suprapubic cystotomy on September 29, 1977. Histologically, it was characterized as transitional cell carcinoma, Grade I to II, with a focal area of Grade IV carcinoma. On November 22, 1977, a large tumor mass on the right side of the bladder neck displacing the rectum posteriorly was noted. The last time the patient was seen on January 31, 1978, the tumor had extended medially to the anterior superior iliac spine.

Cell line SW-1738 was established on September 20, 1977, from the bladder tumor of a 62-year-old male following radical cystectomy. Histopathologically, the tumor was characterized as transitional cell carcinoma, Grade III. Multifocal growth of the primary neoplastic process, invasion, metastases, and recurrence, even after a seemingly successful treatment at a very early stage, are characteristics of this type of neoplasia. In an effort to facilitate the study of the biology of this tumor, investigators have attempted to establish permanent

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Table 1
Morphological characteristics of cell lines in tissue culture

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell morphology</th>
<th>Nuclear characteristics</th>
<th>Multinucleation</th>
<th>Plating efficiency</th>
<th>Doubling time</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW-780</td>
<td>Round or ovoid</td>
<td>Round</td>
<td>±</td>
<td>41</td>
<td>38</td>
</tr>
<tr>
<td>SW-800</td>
<td>Large elongated polygonal or rounded</td>
<td>Round or ovoid, occasionally indented</td>
<td>+</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>SW-1738</td>
<td>Large polygonal</td>
<td>Round, ovoid, occasionally indented, centrally located</td>
<td>±</td>
<td>61.8</td>
<td>32</td>
</tr>
<tr>
<td>SW-1710</td>
<td>Moderately elongated</td>
<td>Round or ovoid</td>
<td>±</td>
<td>62.4</td>
<td>37</td>
</tr>
</tbody>
</table>

N:C, nuclear:cytoplasmic ratio.

and composition of enzyme stains were the same as described previously (18). HeLa cells and mouse cells were run simultaneously as controls. These results were further confirmed by using the method of Halton et al. (9).

Cytogenetic analysis, including banding, on cultured tumor cells was done by methods described by Peterson et al. (20) and Seabright (22).

Nude Mice. Female BALB/c athymic mice (Charles River Breeding Laboratories, Inc., Wilmington, MA) were maintained in a pathogen-free environment. Before use, animals were tested for the presence of LDH virus which affects the ability of the host to clear LDH (21) according to the method described previously (12). Animals used in the present study were found to be free of LDH virus infection.

Collection of Mouse Plasma Samples. For the determination of the amount of human LDH present in the plasma of tumor-bearing mice, blood was drawn by orbital venipuncture with heparinized microcapillary tubes. Plasma was separated by centrifugation and stored at −80°C until assayed.

Solid Tumors in Nude Mice. For injection into nude mice, cultured tumor cells were dispersed with 0.5% trypsin:0.2% EDTA in Hanks' balanced salt solution (GIBCO) and adjusted to 1 × 10⁶ viable cells/0.2 ml. Cell viability was determined by the trypan blue dye exclusion test. Initially, s.c. tumors were established by injecting the animals with 1 × 10⁶ viable tumor cells. In subsequent experiments, tumor propagation was accomplished by transplanting s.c. through a skin incision in the anterior lateral thoracic region a small piece of tumor measuring approximately 0.3 × 0.3 cm (10). Tumor size was measured in 2 dimensions with callipers, and tumor volume was calculated weekly by using the formula

\[ \text{Volume} = \text{Length} \times (\text{width})^2 \times 0.4 \]

Nude mouse-grown tumors were removed, fixed in buffered formalin, embedded in paraffin, and stained with hematoxylin/eosin.

For transmission electron microscopy, tumor tissue was cut into fragments 0.5 to 1 mm thick and fixed in cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, immediately after removal. They were embedded in Spurr, and thin sections were prepared with a Reichert ultramicrotome, stained with uranyl acetate:lead citrate, and examined in a Philips EM-300 electron microscope.

RESULTS

Tissue Culture

The morphological characteristics of the cell lines growing in tissue culture are presented in Table 1, and Table 2 summarizes their enzyme phenotypic profile.

Tumor Growth in Nude Mice

Transplantation of 1 × 10⁶ viable tumor cells s.c. in nude mice resulted in tumor growth for the cell lines SW-780, SW-800, and

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characteristics of nude mouse-grown tumors, solid pieces of first positive evidence of tumor growth, was 3 weeks for line tumor measuring approximately 0.3 x 0.3 cm were transplanteds.c. as described in "Materials and Methods." The follow-up of line SW-1738. Subsequently, in order to establish the growth SW-780, 6 to 8 weeks for line SW-800, and 3 to 4 weeks for SW-1738. The latency period, time between transplantation and grown tumors exhibited similar morphological characteristics. They were arranged primarily in cords supported by the nude mouse were round or ovoid with large, moderately fibrosis was moderate. Local invasion and métastases were cytoplasm. They were arranged primarily in cords supported by the centrally located delicate fibrovascular tissue (Fig. 2). Stromal fibrosis was moderate. Local invasion and metastases were found primarily in the regional lymph nodes and the lungs as reported previously (7). Electron microscopic studies revealed that both cells from tissue culture and those of the nude mouse-grown tumors exhibited similar morphological characteristics. The nuclei were large and rounded with evidence of chromatin clumping and formation of nucleoli. The cytoplasm contained well-developed mitochondria, poorly developed Golgi apparatus, short profiles of rough endoplasmic reticulum, and free ribosomes. Cytoplasmic microfilaments and occasional lipid bodies were also observed. The plasma membrane exhibited numerous well-developed microvilli. Adjacent cells showed infolding and interdigitation of surface microvilli, and desmosomes and tight junctions were present (Figs. 3 and 4).

Cells of line SW-800 were round or ovoid with large, moderately hyperchromatic nuclei and slightly eosinophilic or amphophilic cytoplasm. They were arranged in cords supported by a centrally located delicate fibrovascular tissue (Fig. 2). Stromal fibrosis was minimal. The tumor invaded locally and metastasized primarily in the regional lymph nodes and lungs (11). Electron microscopically, cells of the line SW-1710 were characterized by large nuclei and cytoplasm containing a small number of well-developed mitochondria, short profiles of rough endoplasmic reticulum, few free ribosomes, recognizable Golgi apparatus, underdeveloped smooth endoplasmic reticulum, and occasional microfilaments. Occasional broad-based surface microvilli were also observed.

Repeated attempts to grow tumors in the nude mice by injecting cells of the line SW-1710 failed.

Production of LDH

We further investigated the ability of all 4 tumor cell lines to produce LDH under different environmental conditions. LDH was present in measurable amounts in tissue culture media, cell extracts, and the serum of tumor-bearing nude mice. Fig. 5 shows the LDH isoenzyme profile under varying conditions, and quantitation of LDH isoenzymes in the systems studied is presented in Table 3.

Karyotyping and Chromosome Analysis

Tumor Line SW-780. A number of karyotypes of the cell line were prepared. None of the metaphases had a normal No. 4 chromosome. The No. 3 chromosomes were usually aberrant but not in a consistent way in that the loss of material seemed not to be consistent from chromosome to chromosome. Multiple copies of some normal chromosomes were present in this submetaploid cell line, notably chromosomes 5, 12, 13, 19, and 22. There were 2 identifiable marker chromosomes present, m1 and m2. m1 is a (4p), while m2 most likely is an (5q). In addition, a number of unidentifiable marker chromosomes were present in all karyotypes studied (Fig. 6).

Tumor Line SW-800. There were 5 karyotypes studied. All karyotypes prepared were XY. All normal chromosome groups were represented with some random loss and gain of normal chromosomes; trisomy 14 was observed in 3 of 5 karyotypes studied. In all karyotypes, a marker chromosome, m1, which may have chromosome 21 involvement, was present. A large subtelocentric chromosome was seen in 2 of the 5 karyotypes. Unidentifiable marker chromosomes ranging from 1 to 5 were

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PGM1</th>
<th>PGM3</th>
<th>ESD</th>
<th>Me-2</th>
<th>AK-1</th>
<th>GLO-1</th>
<th>G6PD</th>
<th>LDH</th>
<th>Phenotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW-790</td>
<td>1</td>
<td>1-3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>B</td>
<td>Human</td>
<td>0.014</td>
</tr>
<tr>
<td>SW-800</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>B</td>
<td>Human</td>
<td>0.0018</td>
</tr>
<tr>
<td>SW-1738</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>B</td>
<td>Human</td>
<td>0.003</td>
</tr>
<tr>
<td>SW-1710</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1-2</td>
<td>1</td>
<td>1</td>
<td>B</td>
<td>Human</td>
<td>0.032</td>
</tr>
</tbody>
</table>

PGM, phosphoglucomutase, EC 2.7.5.1; ESD, esterase D, EC 3.1.1.1; Me-2, malate dehydrogenase, EC 1.1.1.40; AK-1, adenylic kinase, EC 2.7.4.3; GLO-1, glucose I, EC 4.4.1.5; G6PD, glucose-6-phosphate dehydrogenase, EC 1.1.1.49; LDH, EC 1.1.1.27.6 Isoenzyme profiles of cell lines SW-800 and SW-1738 are in accord with those reported previously (19, 23).
seen in all 5 karyotypes (Fig. 7).

Tumor Line SW-1738. All 6 karyotypes prepared had a single X chromosome. Of the normal chromosomes, chromosome 14 appeared to be underrepresented. There were 2 marker chromosomes, m1 and m2, found in all karyotypes. m1 is an 11 p"q". m2 appears to be an i(17q). Unidentifiable marker chromosomes were found in all 6 karyotypes studied (Fig. 8).

Tumor Line SW-1710. There were 4 karyotypes prepared for the present study. Of the normal chromosomes, 11, 13, and 15 seemed underrepresented. Five marker chromosomes were found in each karyotype. m1 is a 6q+ m2 is an i(17q), and m3 is a 13q+*. The component parts of marker chromosomes m4 and m5 could not be identified. A number of unidentifiable marker chromosomes ranging from 15 to 30/karyotype were also observed (Fig. 9).

Table 4
Chromosome frequency distribution

<table>
<thead>
<tr>
<th>Cell line</th>
<th>No. of metaphases</th>
<th>No. of chromosomes precisely counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW-790</td>
<td>1</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>87</td>
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<td></td>
<td>4</td>
<td>88</td>
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<tr>
<td></td>
<td>1</td>
<td>89</td>
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<tr>
<td></td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td>SW-800</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>49</td>
</tr>
<tr>
<td>SW-1738</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>66</td>
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<tr>
<td></td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>114</td>
</tr>
<tr>
<td>SW-1710</td>
<td>3</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>74</td>
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<td></td>
<td>6</td>
<td>75</td>
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<td></td>
<td>1</td>
<td>79</td>
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<tr>
<td></td>
<td>1</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>83</td>
</tr>
</tbody>
</table>

* Of 100 metaphases scanned for ploidy, 5 had chromosome counts in the high-40 chromosome range, and 95 had chromosome counts of 80 to 90. Of 100 metaphases scanned for ploidy, 90 had 46 to 50 chromosomes, and 10 had 90+ chromosomes.
* Of 100 metaphases scanned for ploidy, 85 had 60+ chromosomes, and 15 had 100+ chromosomes.
* Of 100 metaphases scanned for ploidy, all were in the range of 70 to 90 chromosome counts.

Table 3
LDH isoenzyme profile of cell lines SW-780, SW-800, SW-1738, and SW-1710

<table>
<thead>
<tr>
<th>Source</th>
<th>LDH-5</th>
<th>LDH-4</th>
<th>LDH-3</th>
<th>LDH-2</th>
<th>LDH-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A B C D</td>
<td>A B C D</td>
<td>A B C D</td>
<td>A B C D</td>
<td>A B C D</td>
</tr>
<tr>
<td>Culture medium</td>
<td>0 2.3 7 17.7</td>
<td>0 6.7 14 27</td>
<td>9.4 42.9 36 34.9</td>
<td>25.6 43.4 23.3 20.3</td>
<td>65 4.7 18.7 0</td>
</tr>
<tr>
<td>Cell extract</td>
<td>1.2 4.6 9.6 8.5</td>
<td>8.2 23.3 23.7 16.2</td>
<td>28.2 36.0 37.5 45</td>
<td>32.3 27.4 22.6 30.3</td>
<td>30.1 9.7 6.5 0</td>
</tr>
<tr>
<td>Serum of tumor-bearing mice</td>
<td>2 4.4 5.1</td>
<td>64.8c 77.7 77.7</td>
<td></td>
<td></td>
<td>20.4 15.0 14.7 12.8 2.9 2.5</td>
</tr>
<tr>
<td>Control</td>
<td>5.1</td>
<td>3.8</td>
<td>17.7</td>
<td>42.8</td>
<td>30.6</td>
</tr>
</tbody>
</table>

a, cell line SW-780; B, cell line SW-800; C, cell line SW-1738; D, cell line SW-1710.

a 100x concentrate.

densitometric separation of LDH-4 and LDH-3 was not clear due to the mouse LDH-5 between them. The values presented here represent both isoenzymes.
Chromosomal analysis of nude mouse-grown tumors was not performed in the present study.

Table 4 summarizes the chromosome frequency distribution of the cell lines under study.

DISCUSSION

In this study, light and electron microscopic observations showed a close morphological and structural similarity for the same tumor cell line under different environmental conditions. It is worth emphasizing the fact that tumors grown in the nude mouse exhibited characteristics identical with those of the tumor of origin, regardless of transplant generation (Figs. 1 to 4), an observation further corroborating previous reports on this subject (7, 12, 13). Cytogenetic studies revealed a number of marker chromosomes consistently reproducible in many karyotypes of the same cell line. An interesting finding was the identification of a subtelocentric marker chromosome seen in cell line SW-800 which has been observed in earlier studies. This observation may indicate that certain marker chromosomes are relatively stable, and their presence may be used in identifying individual tumors and, to a certain degree, predicting their biological behavior (4, 5). More recently, monosomy of chromosome 9 was described as a nonrandom abnormality in bladder tumors (8). Although we have observed this in a number of bladder cancers, none of the cell lines under discussion presented with this abnormality. From Table 4, it becomes apparent that 3 of our cell lines showed a bimodal chromosome ploidy distribution. Whether this is an indication of different cell clones in the same cell line is subject to further investigation. Of interest was the absence of X chromosome from the line SW-1710 in all karyotypes studied. Although this observation is somewhat disconcerting, we know that the X chromosome is present in some form, since the gene regulating glucose-6-phosphate dehydrogenase is on the X chromosome. We suspect that the X chromosome is broken in 2 parts, one of which may be on the q arm of m1 and the other on the q arm of m3.

The different electrophoretic mobilities of human and mouse LDHs (12) make the former a useful marker in identifying the human nature of any tumor grown in the nude mouse. A comparison of LDH profiles of the various cell lines, as shown in Table 2, indicated that cell line SW-780 was characterized by a very low concentration of LDH-5 and LDH-4 isoenzymes in culture media and cell extracts, whereas LDH-1 isoenzyme was the dominant fraction on both media. In contrast, SW-1710 showed no LDH-1 in culture media or cell extracts. However, traces of LDH-1 in a concentration below the detection level could be seen only in the fraction of cell extracts when gel troughs were loaded with 4 or more µl of 100 x concentrates of cell lysates. As noted previously, mouse LDH-5 moves electrophoretically between human LDH-4 and LDH-3 (8, 9), and quite frequently, in a mixture of mouse and human LDHs, overlapping of isoenzymes of different origin may be observed in that region. This is most probably the reason for the high values of LDH-4 in the serum of tumor-bearing animals.

It is of interest that line SW-1710 failed to grow when transplanted in the nude mouse regardless of the number of injected cells. All attempts to modify the host by exposing the recipient mice to whole-body radiation (500 rads) or treating the animals with corticosteroids or antilymphocytic serum were unsuccessful.

In conclusion, the described tumor lines of human bladder transitional cell carcinomas showed morphological, biological, and biochemical characteristics similar to those of the original tumors in a consistent pattern regardless of the number of tissue culture or nude mouse passages. Their enzyme phenotypic profile along with the karyotyping made them distinct from any other cell line. In addition, their ability to produce tumors when transplanted into nude mice may facilitate additional studies aiming at clarifying the multifaceted biology of this type of human urothelial neoplasia. Furthermore, additional studies of line SW-1710 could contribute to better understanding of those factors that may be operative in controlling tumor growth in the nude mouse.

REFERENCES


*J. Fogh, personal communication.

*W. D. Peterson, personal observations.
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Fig. 1. Original tumor, SW-780: slightly elongated, transitional epithelial cells arranged in papillary forms and supported by a delicate fibrovascular core. H & E, × 250.

Fig. 2. Tumor SW-780 grown s.c. in the nude mouse. The tumor is characterized by elongated cells resembling transitional epithelium forming papillary structures supported by a delicate fibrovascular core (compare with Fig. 1 showing the original tumor). H & E, × 250.

Fig. 3. Electron micrograph showing SW-780 cells growing in tissue culture. The cells are characterized by well-developed surface microvilli, large round or slightly indented nuclei, and poorly developed endoplasmic reticulum. Uranyl acetate:lead citrate, × 9000.

Fig. 4. Electron micrograph showing a portion of SW-780 tumor growing s.c. in the nude mouse. The neoplastic cells are characterized by well-developed surface microvilli, round nuclei, and poorly developed rough-surfaced endoplasmic reticulum (compare with Fig. 3). Uranyl acetate:lead citrate, × 9000.

Fig. 6. Karyotype of cell line SW-780 in a subtetraploid range with 2 identifiable marker chromosomes. Note absence of chromosome 4.

Fig. 7. Karyotype of cell line SW-800. All karyotypes studied were XY. Note a large subtelocentric marker chromosome (arrow).

Fig. 8. Karyotype of cell line SW-1738 with 2 identifiable marker chromosomes, Y-chromosome is missing.

Fig. 9. Karyotype of cell line SW-1710 showing a number of identifiable and unidentifiable marker chromosomes.
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