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The rat bladder tumor model system RBT resembles phenotypically and cytogenetically human superficial transitional cell carcinoma

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Summary. A cohort of 300 ACI rats was kept under standard laboratory conditions. After 30 months or upon natural death, complete autopsy was performed. In the genitourinary tract four kidney and five bladder tumors were found. Two of these bladder tumors, RBT323 and RBT157, are serially transplantable. In the fifth transplant generation the RBT323 tumor becomes metastatic to the lungs in more than 90% of animals. The metastatic ability of the RBT157 tumor changes from low to intermediate (50% of the rats have lung metastases) in the fourth passage. Histologically, the initial passages of the RBT323 and 157 tumors are grade II transitional cell carcinoma (TCC). The histological pattern of the RBT157 tumor remains essentially unchanged, whereas the RBT323 tumor progresses to a grade III tumor in the third passage. Electron microscopical studies reveal oblong elliptical and round vesicles lined by an asymmetrical unit membrane in the tumor cells, which stresses the urothelial origin of the tumors. Immunohistochemically both tumors show expression of cytokeratin 5, 7, 8 and 18. The progression of the tumors to a metastatic phenotype, however, is not associated with a specific change in the morphological characteristics. Cytogenetic analysis shows that both tumors are peridiploid with few marker chromosomes. Interestingly, both of these independently arising tumors exhibit a loss of chromosome 5. Rat chromosome 5 is syntenic to the major portion of human chromosome 9 (p23-qter). Loss of chromosome 9 is a cytogenetic trait of human superficial TCC, hence the RBT model is also in cytogenetic respect similar to human TCC. Two independently arising rat tumor lines that initially resemble superficial TCC both phenotypically and cytogenetically are described. Upon serial transplantation both lines progressed to a more aggressive tumor, albeit to a different extent (highly vs moderately metastatic). Thus this model system may be helpful in the identification of specific markers associated with the progression of superficial bladder cancer.

Key words: Transitional cell carcinoma – Tumor progression – Rat tumor model – Marker chromosomes

Estimates of new cancer cases in the United States show that cancer of the bladder is the fourth most prevalent cancer in males and the ninth in females. Bladder cancer is about three times as common in men as in women. The USA estimates for 1990 are 36000 new cases in males and 13000 new cases in females. Bladder cancer death rates have been fairly constant for the past 60 years [38, 39]. Recurrence rates vary from 30% for solitary papillary tumors to more than 90% in some cases of multiple tumors. Some 5–30% of all cases exhibit progression of disease [28]. In general tumor progression is associated with large tumor size, advanced stage, high grade, multiplicity of tumors, vascular or lymphoid invasion and concurrent carcinoma in situ [28, 33]. More importantly, recent studies indicated involvement of specific chromosomal changes in tumor progression of transitional cell carcinoma (TCC). In low-grade, noninvasive TCC, trisomies for chromosome 1, 7 and 11 and monosomy for chromosome 9 were found; the farther the tumors had progressed, the greater the number of chromosomal aberrations [14]. Allelic losses were found for chromosome 9, 11 and 17 [44]. The greatest frequency was seen for chromosome 9q (67% of the informative cases) and 17p (63% of the informative cases). The authors concluded, after having investigated more cases, that the loss of chromosome 17 is a late event in tumor progression and can be used to distinguish between low- and high- grade TCC [26].

An important restriction in the study of human bladder cancer is the lack of available tumor tissue for experimental purposes. In particular, tumor material truly representative for the progression from low- to high-

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stage TCC is very difficult to obtain. The tumor volumes obtained from routine biopsies and cystectomies are often small and damaged due to intra-operative or prior treatment procedures. Many important factors not specific for bladder cancer cannot be controlled with clinical material, including genetic differences, age, time of initial onset of the disease and variability of cell types. To overcome these problems a variety of animal models for bladder cancer have been developed [1, 30].

After heterotransplantation of human bladder cancer tissue into immune-deprived and nude mice, some short-term and serially transplantable xenograft lines have been established. No correlation has been found between the success of xenografting and the grade of the "donor" tissue [20, 30, 43]. Russell et al. demonstrated substantial heterogeneity within and between different TCC xenografts. Studies with these lines may help to explain the diverse natural history of bladder cancer [34].

Animal tumor models can be divided into carcinogen-induced and spontaneous tumor models. A number of potent carcinogens can produce bladder neoplasms in laboratory animals [1, 5, 30]. In most induced tumors concurrent squamous metaplasia occurs, and most tumors consist of both poorly differentiated TCC and squamous cell carcinoma (SCC) [5]. Recently, Steinberg and associates reported on the fact that upon NMU treatment, bladder tumors could be induced in Fischer 344 rats without the occurence of squamous carcinoma. Surprisingly, however, not in all animals (3/6) were cytogenetic changes found in these tumors [42]. Few spontaneous bladder tumor models are available up to now. The Brown Norway rat has a high incidence of spontaneously occurring urothelial tumors of the bladder and ureter; 35% of the male and 3% of the female animals develop bladder tumors, which are transplantable in syngeneic rats [3]. The invasive tumors consist of transitional epithelium, but also areas of squamous metaplasia are observed; SCC, however, is not common. In the Nb rat a single transplantable transitional cell tumor has been described [9]. In DA/Han rats 54% of male and 14.4% of female animals develop spontaneous urinary bladder tumors [8]. The incidence increases with age; also for induced carcinomas an increased risk of urinary bladder carcinogenesis with age has been reported [10]. The most frequent tumors found in the DA/Han rats are solid type TCC; 0.3% of the male and 9.6% of the female rats have SCC [8]. None of these models has been used to study the progression of bladder cancer.

ACI rats are known for their spontaneous genitourinary carcinomas, especially prostatic carcinomas, but TCC has been described [22, 46]. In this paper we present the characterization of two bladder tumor lines transplantable in ACI rats. These lines were evaluated for phenotypic and genetic changes. Serial transplantation was used as a model to study the progression of the lines to more aggressive phenotypes.

Materials and methods

Animals

For the initial tumor-finding study, 300 male ACI/SeqHSD (Harlan Sprague Inc., Indianapolis, Ind.) were housed in an environmentally controlled room and were provided with food and water ad libitum. After 30 months or upon natural death, complete autopsy was performed. The urinary bladder and prostate were excised in toto and fixed for at least 24 h and embedded in paraffin. Sections were cut transversely through the midportion of the bladder, and four 5-μm sections were taken from each half to adequately sample the entire bladder and prostate; these sections were stained with hematoxylin and eosin.

Tumor transplantation

Primary tumors and tumors from subsequent passages were excised from tumor-bearing animals; after removal of normal and necrotic tissue, the tumor tissue was cut into pieces of 20 mg. A 0.5-cm incision was made in the right flank of recipient animals, and after separating the subcutaneous tissue by blunt dissection, the tumor fragment was placed subcutaneously (s.c.). The incision was closed using skin clips (7.5 × 1.75 mm, Aesculap, Germany). In this way a tumor take of 100% was achieved. Per passage at least six rats were implanted. Tumor growth was followed by measurements of tumor size according to the method of Janik et al. [16]. At least once per week, length (l), width (w) and height (h) were measured using a slide caliper. No correction was made for skin thickness. The tumor volume (V) was calculated from the formula $V = 0.5236 \times l \times w \times h$. Per animal, the paired serial measurements of days post-tumor inoculation (x-axis) and log tumor volume (y-axis) were plotted to reveal the period of exponential growth. By means of linear regression the best lines were fitted. The slopes of the regression lines are proportional to the growth rate; the inverses of the slopes represent the tumor doubling times.

Light and electron microscopy

When the subcutaneously growing tumors reached a size of 5–10 cm^3, rats were killed with an ether overdose and complete necropsy was performed. Both lungs and representative pieces of the tumor were routinely fixed in 4% buffered formalin for at least 24 h and embedded in paraffin. Other organs were grossly inspected for metastases and, if suspicious, also fixed in formalin. To adequately sample the tumors and the lungs, four 5-μm sections were taken and stained with hematoxylin and eosin. Tumors were assessed according to Squire [40] and Koss [18].

For electron microscopy representative parts of the tumors were cut into small pieces and prefixed with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 C, and postfixed in 0.1 M palade buffer. After washing in the same buffer and degradation in graded ethanols, the specimens were embedded in Epon 812. Thin sections were double-contrasted with uranyl acetate/lead citrate and examined in a Philips EM 300 [45].

Immunohistochemistry

For immunohistochemical studies we used the indirect immunofluorescence technique as described before [4]. Frozen sections of the tissues (6 μm) were cut on a cryostat and air-dried before fixation in methanol (5 min, −20°C) and acetone (three times 5 s). After being washed in phosphate-buffered saline (PBS) for 10 min, the sections were incubated with 20% normal rat serum (NRS) in PBS at room temperature (30 min), and subsequently with the primary antiserum

for 60 min. After repeated washing in PBS (three times 10 min) the rabbit anti-mouse conjugated to peroxidase (DAKOpatts, Denmark) diluted 1:100 (v/v) in PBS with 20% NRS was applied for 30–45 min. After repeated washings in PBS (three times 10 min), peroxidase activity was detected with 3-3'-diaminobenzidine (DAB; 6 mg/10 ml, 0.65% imidazole in PBS) and hydrogen peroxide to a final concentration of 0.01%. After incubation for 5 min and extensive washing with tap water, the slides were counterstained with hematoxylin and mounted with Permount (Fisher Scientific, N.J.).

The following antibodies directed against intermediate filament proteins were used in this study:

1. The mouse monoclonal antibody RCK 102 directed against cytokeratins 5 and 8 (nomenclature according to Moll [23]). This antibody stains virtually all epithelial tissues, but not nonepithelial tissues [32].

2. The mouse monoclonal antibody RCK 105 directed against cytokeratin 7 and reactive with a subgroup of glandular epithelial tissues [32].

3. The mouse monoclonal antibody RGE 53 directed against cytokeratin 18, which specificaly recognizes columnar epithelial cells from digestive, respiratory, and urogenital tracts, endocrine and exocrine tissues, and mesothelial cells. No significant reaction is normally found in squamous epithelial or nonepithelial tissues [31].

4. The mouse monoclonal antibody RKSE 60 directed against human skin cytokeratin 10 and specific for keratinizing stratified squamous cells. No reaction is found with this antibody in columnar epithelial cells, nonkeratinizing squamous cells, or nonepithelial cells [31].

5. The mouse monoclonal antibody RV 203, specific for vimentin [13].

6. The mouse monoclonal antibody RD 301, specific for desmin [29].

Soft-agar culture

When tumors reached a size of 5–10 cm^3 the rats were killed and tumor material was suspended in RPMI 1640 tissue culture medium (Gibco, Paisley, UK). After careful removal of normal tissue and areas of tumor necrosis, tumors were cut into pieces and minced with scissors into a 300-μm metal sieve and continuously washed with RPMI 1640 solution into a petri dish. The minced tumor tissue was passed through a 40- to 70-μm nylon filter (Ortho Diagnostics, Beerse, Belgium) to obtain a single cell suspension. The single cell suspension was centrifuged at room temperature at 400 *g* for 5 min, after which the supernatant was discarded. Upon resuspension of the cell pellet in double-enriched CMRL 1066 (Gibco, Paisley, UK), cell density and viability were determined by adding 15 μl trypan blue solution (25 mg in 5 ml 0.3% acetic acid) to 15 μl cell suspension and simultaneously counting colored and not colored cells using a Bürker-Türk hemocytometer. For the detection of the in vitro growth potential a modified double-layer soft agar culture method as originally described by Salmon and Hamburger [11] was used [2]. Tumor cell suspensions were plated in the upper layer of the two-layer culture system in a range of 10000–500000 cells per dish. Cells were cultured immediately after preparation of the single cell suspension; growth potential was quantified using an Omnicon Fas II automated colony counter (Milton Roy, Rochester, N.Y. [12]).

Chromosomal analysis

Single cell suspensions were made by mincing tumors with scissors in RPMI 1640 culture medium containing 10% fetal bovine serum (FBS) 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 fifty metaphases were counted for each tumor. Chromosomes were banded using the trypsin-Giemsa technique [37] and arranged according to the scheme of Satoh et al. [35]. At least five G-banded metaphases were karyotyped for each tumor.

Results

Etiology and growth characteristics

300 male ACI rats were kept under standard laboratory conditions, without influence of any known carcinogen. Approximately 150 animals died within 24 months; the remaining animals were killed at 30 months. In the genitourinary tract four kidney tumors and five bladder tumors were found. In contrast to other studies, no prostate tumors developed in these rats [46]. All five bladder tumors were transplanted s.c. in syngeneic rats; two tumors appeared to grow under these conditions. These tumors were designated rat bladder tumors 323 and 157 (RBT323 and RBT157). Initially both tumors typically grew as a cyst, with papillary tumors inside this cyst. Due to this growth pattern the tumor doubling times of the first passages should be considered inaccurate. Upon further passaging the tumor acquired a more solid growth pattern. The tumor doubling time of the RBT323 tumor decreased from 13 days in the second passage to 3.5–4 days in the eighth and subsequent passages. The tumor doubling time of the RBT157 tumor, however, remained almost unchanged. In the first passage the doubling time was 11 days, while subsequent passages had doubling times of 8–9 days.

The metastatic capacity of both tumors was low in the initial passages (i.e. less than 5% of the animals had microscopic lung metastases) (Table 1). However, in passage 5 for the RBT323 tumor and passage 4 for the RBT157 tumor, both tumors acquired higher metastatic potential, especially to the lungs. More than 90% of the RBT323-bearing animals had microscopic and/or macroscopic lung metastases; occasionally an axillary or retroperitoneal lymph node metastasis was found. No liver metastases were found. About 50% of RBT157-bearing animals showed metastases to the lungs.

Cells of transplant generation 1, 12 and 13 of the RBT323 tumor and generation 1 and 4 of the RBT157 tumor were tested for their in vitro growth potential. The trypan blue exclusion test usually showed viability of 20%. At 14 days after plating of 500000 RBT323 cells, 250–300 colonies were formed in the dishes, i.e. plating efficiency was 0.06%. Cells of RBT157 passage 1 formed 20 colonies, while cells of transplant generation 4 formed 70 colonies after 14 days of culture (plating efficiency 0.004 vs 0.014%).

Table 1. Growth characteristics of rat bladder tumor lines

Tumor passage	Tumor doubling time[a]	Metastases[b]
RBT323	ND	0/1
Passage 1	ND	0/6
Passage 2	13.4 (1.4)	0/8
Passage 3	11.8 (1.2)	1/10
Passage 4	5.9 (1.0)	0/9
Passage 5	5.7 (0.3)	3/7
Passage 6	4.4 (0.1)	11/13
Passage 8	3.8 (0.2)	15/15
Passage 10	3.7 (0.2)	9/9
Passage 12	4.2 (0.3)	19/22
Passage 13	3.3 (0.3)	6/6
Passage 15	4.3 (0.3)	5/6
RBT157	ND	0/1
Passage 1	11.2 (0.8)	0/4
Passage 2	ND	1/7
Passage 3	10.5 (1.1)	2/9
Passage 4	8.2 (0.3)	5/8
Passage 6	9.5 (1.8)	2/6

[a] Tumor doubling time in days, standard error of the mean in brackets (*ND*, not determined)
[b] Number of rats with lung metastases/total number of rats implanted with respective tumor passage

Histology

The original RBT323 tumor showed a multicystic growth pattern with solid, trabecular and papillary regions (Fig. 1). The cells were slightly disorderly and the nuclei moderately atypic. There was increased mitotic activity, 0–2 mitoses per high-power field (HPF; 40×); grade II TCC. The primary tumor exhibited no invasive growth. The wall of the cyst was lined by a three- to seven- cell-layer thick transitional epithelium with a superficial layer of umbrella cells. These cells were generally similar to the tumor cells.

The tumor of the first transplant generation had a similar growth pattern; however, invasive growth into the subcutaneous tissues was found. In the subsequent transplant generations the anisokaryosis of the nuclei increased and nuclei became bigger and more disorderly in arrangement, i.e. a grade III transitional tumor. This grade III growth pattern remained the same for all further transplant generations (up to generation 20 at the time of reporting). The RBT157 tumor had generally the same growth pattern as the RBT323 tumor, but had larger cells due to a greater amount of cytoplasm (Fig. 1a–f). The nuclei were moderately atypical. In subsequent transplant generations the tumor remained a grade II TCC. The mitotic index, however, increased to 0–3 per HPF. No squamous differentiation was found in either tumors.

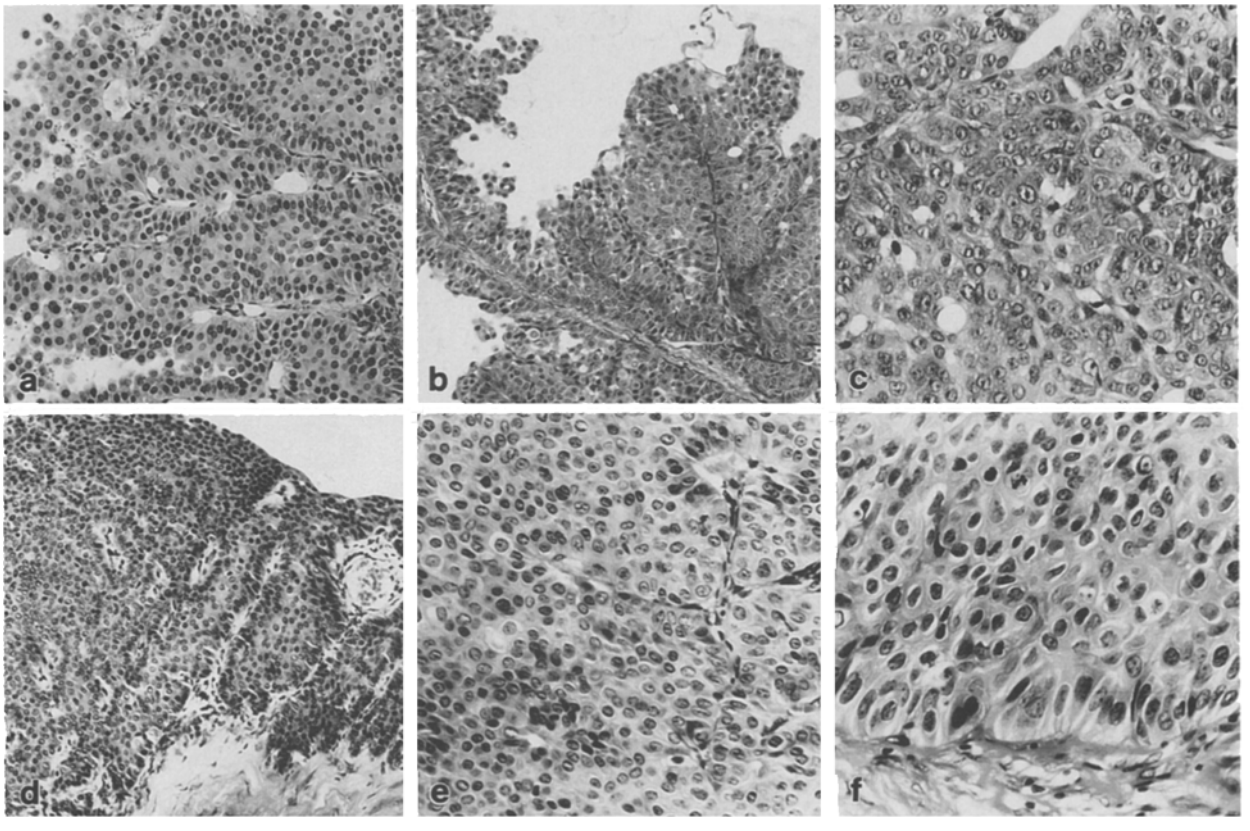

Fig. 1a–f. RBT323 and RBT157 tumors (H&E stain). **a** RBT323; original tumor (×125); **b** RBT323: passage 1 (×125); **c** RBT323: passage 14 (×250). **d** RBT157: original tumor (×125); **e** RBT157: passage 1 (×250); **f** RBT157: passage (4 ×250)

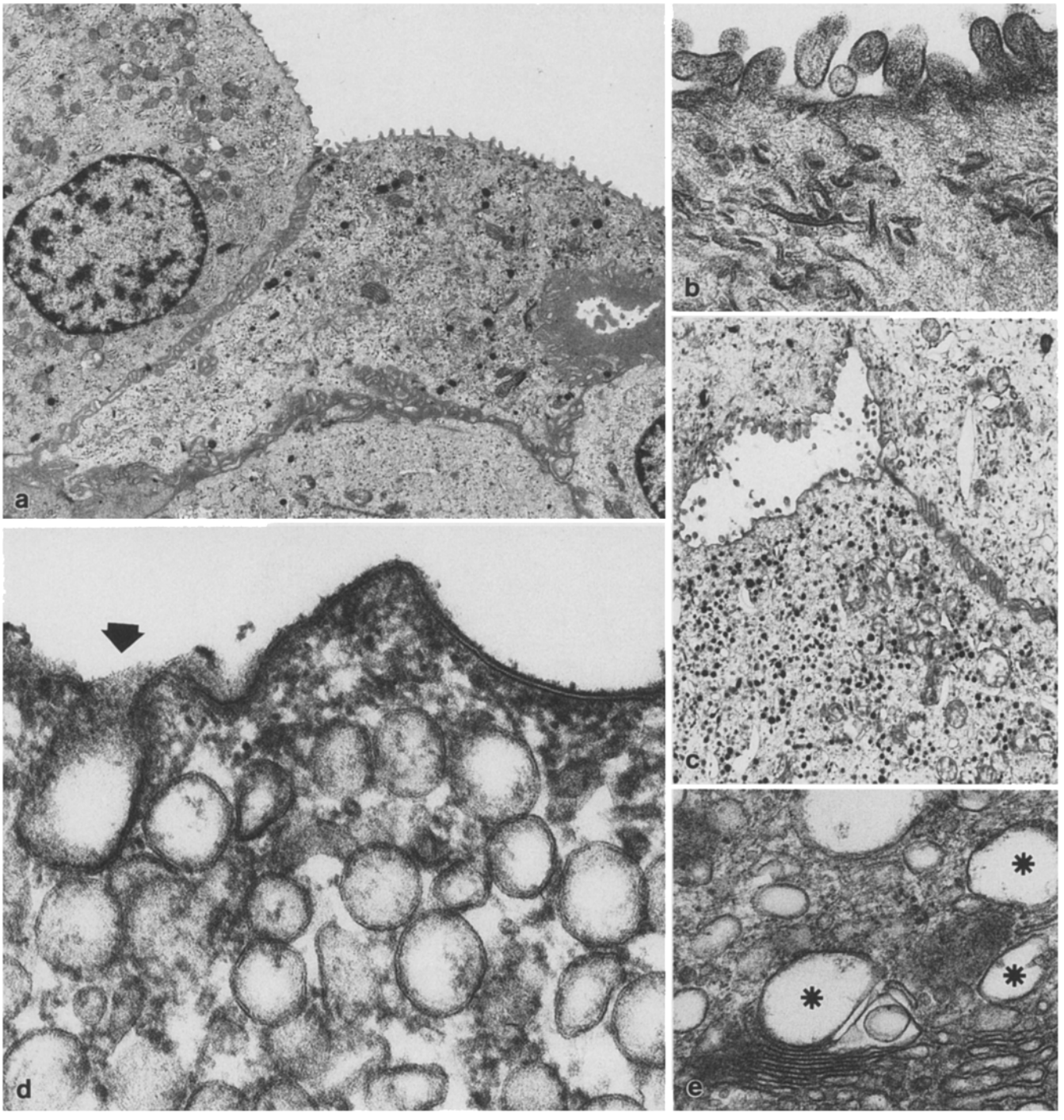

Fig. 2a–e. Electron micrographs of RBT323 tumor. **a** Passage 1 (×5000). **b** Passage 1: microvili and oblong elliptical vesicles (×40000). **c** Passage 1: cell with (neuro-)endocrine granules (×7000). **d** Passage 9: cell with round vesicles lined by an asymmetrical unit membrane (×120000); *arrow* indicates attachment of a vesicle to the luminal membrane. **e** Passage 9: Golgi apparatus with formation of round vesicles (*asterisks*) (×55000)

Electron microscopy

Generally the tumor cells of all passages demonstrated well-developed nuclei and nucleoli, abundant organelles, and distinct junctional complexes (Fig. 2a–e). The basal compartment cells of the first passage of the RBT323 tumor were surrounded by an intact basal lamina. In the intermediate layers many cells showed additionally particles and clusters of glycogen, while other cells contained characteristic electron-dense membrane-bound granules. In some foci, well-developed intermediate sized filaments

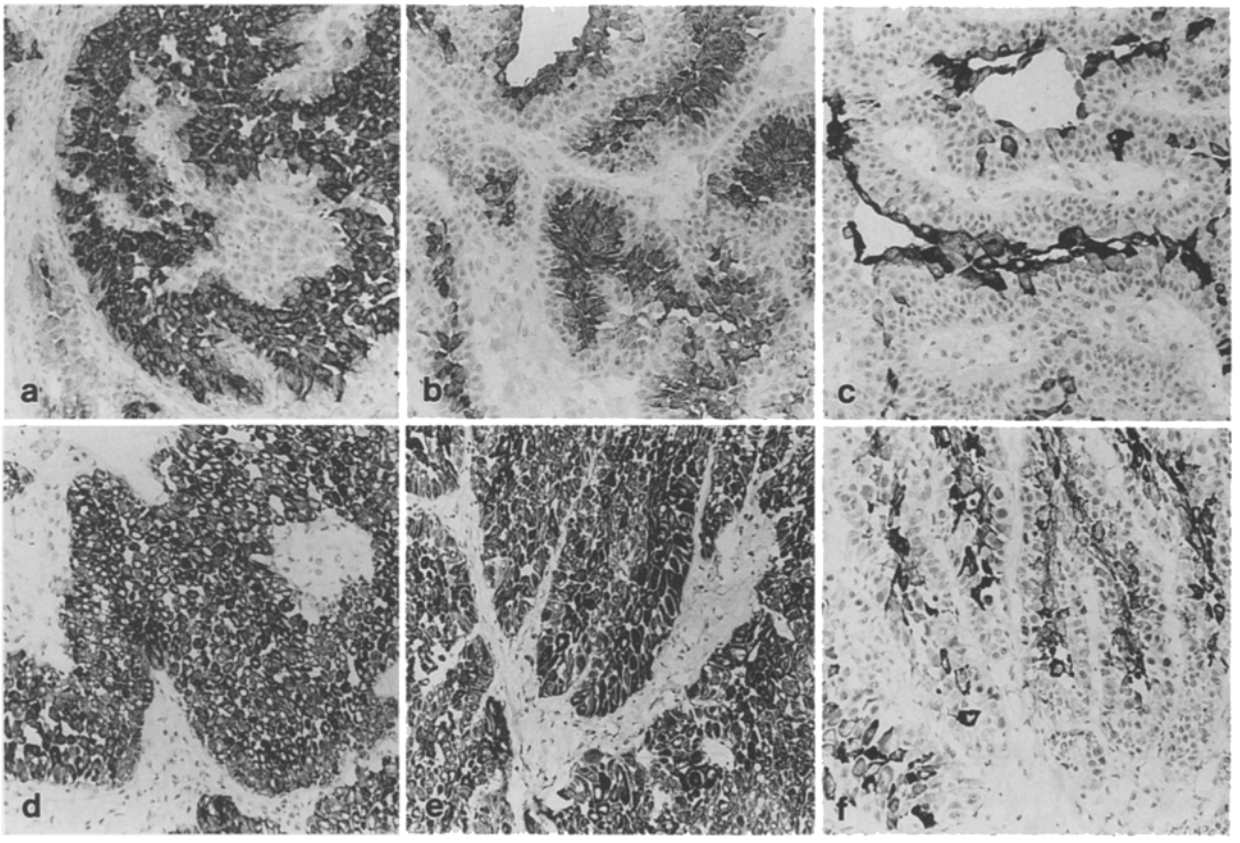

Fig. 3a–f. Immunoperoxidase staining pattern of frozen sections from RBT323 and RBT157 tumors. **a** RBT323: original tumor, stained with anti-cytokeratin MoAb RCK 102 (×125); **b** RBT323: passage 11, RCK 102 (×125); **c** RBT323: passage 1, RGE 53 (×125); **d** RBT157: original tumor, RCK 102 (×125); **e** RBT157: passage 4, RCK 102 (×125); **f** RBT157: passage 4, RGE 53 (×125)

were present in the tumor cells. Numerous cells contained flattened discoid-like or fusiform membrane-bound vesicles, predominantly located in the juxtanuclear and apicoluminal parts of the cytoplasm and in close contact with the cell boundary. An adenomatous growth pattern of tumor cells could be observed, and inter- as well as intracellular luminal formations were present. In the superficial cell layers several cells also featured many flattened vesicles in the apical side of the cell. At higher magnifications the membranes of these vesicles were asymmetric with a thicker fuzzy luminal layer. Upon passaging of the RBT323 tumor the amount of junctional complexes apparently decreased with local absence of the basal laminae. The intracellular flattened vesicles changed to rounder shapes; some of them were obviously attached to the luminal cell membrane. Generally the first passages of the RBT157 tumor showed the same growth pattern as the early passage of the RBT323 tumor. In contrast to the latter, the growth pattern showed no distinct changes in the later passages.

Immunohistochemistry

The normal rat bladder, the original tumors, and tumor tissues of transplant generations 1 to 11 for the RBT323 tumor and generations 1 to 5 for the RBT157 were examined for their intermediate filament expression by using monoclonal antibodies against cytokeratins, vimentin and desmin.

The broadly cross-reacting cytokeratin monoclonal antibody RCK 102 stained all urothelial cell layers of the normal bladder and the RBT157 tumors (Fig. 3d, e). In the RBT323 tumor, however, only the superficial "luminal" cell layer and the directly underlying cell layer were stained (Fig. 3a, b). Antibody RCK 105 also stained all cell layers of the normal rat bladder and the RBT157 tumors. In the primary RBT323 tumor this antibody stained all cell layers except most basal cells (only 10% of the basal cells stained). In tumors of further transplant generations only the superficial cells and the directly underlying cell layer stained. Antibody RGE 53 reacted with the superficial and intermediate cell layers of the normal bladder and with 20% of the basal cells. In the primary RBT157 tumor all superficial and intermediate cell layers and about 30% of the basal cells were stained by RGE 53; in further transplant generations only the superficial and adjacent cells stained (Fig. 3f). In the primary RBT323 tumor all superficial and most intermediate cell cells reacted with RGE 53. Upon transplantation of the tumor generally only superficial cell stained with RGE 53 (Fig. 3c). From passage 1 to passage 6 the proportion of

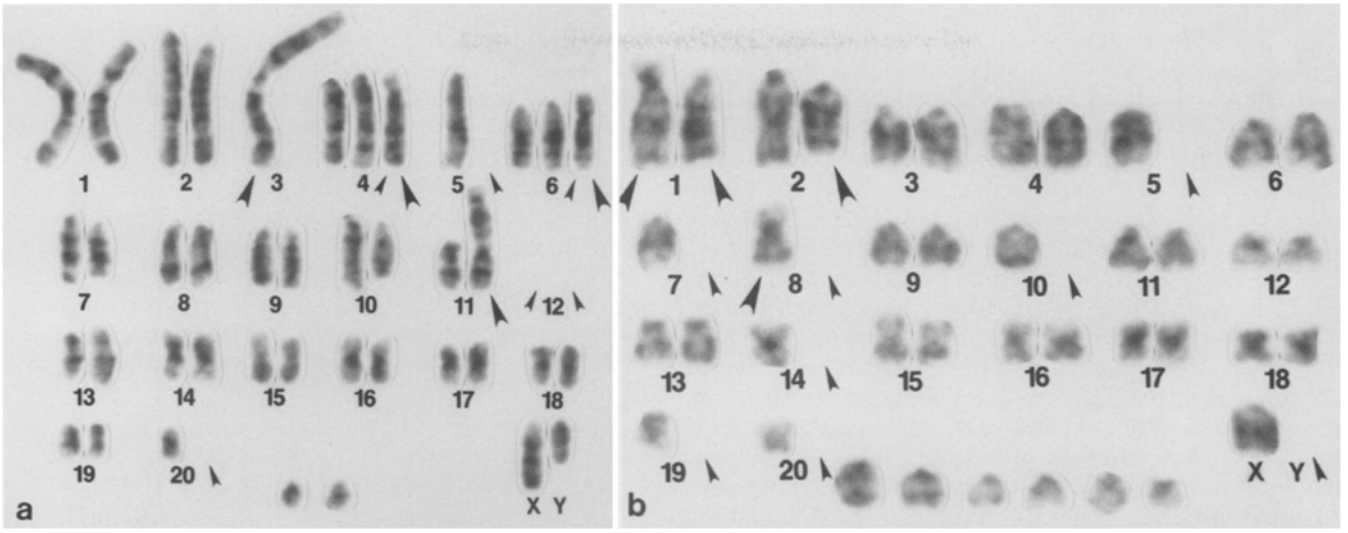

Fig. 4. Cytogenetic analyses of RBT323 and RBT157 tumors. **a** Representative karyotype of RBT157, passage 4 (41, XY, +4, −5, +6, −12, −20, iso(3q), 4p+, 6p+, 11p+, two unknown markers). **b** Representative karyotype of RBT323, passage 7 (40, X, −Y, −5, −7, −8, −10, −14, −19, −20, t(?::1q22→1qter), t(?::1p11→1q54), del(2)q43, 8p+, six unknown markers. Cytogenetic changes are indicated by arrowheads: the *small arrowheads* indicate numerical changes, the *large arrowheads,* structural changes. *Bottom line,* marker chromosomes whose origins are unknown

positive superficial cells decreases from 60% to 10%. This figure increases, however, to 50% in the 11th passage, while in passage 9 all superficial cells are positive.

RKSE 60, an antibody recognizing markers for keratinization, was negative in the normal rat bladder and in the RBT157 tumors. In the RBT323 tumor, cells in the superficial cell layer occasionally stained; the proportion of positive cells was usually only 1% or less. RV 203 detected normal and tumor stroma, but never stained normal rat urothelium or tumor tissue of both tumors. Neither normal rat bladder nor tumor tissues stained with RD 301.

Chromosomal analysis

For cytogenetic studies the tumors were transplanted in female rats; the existence of the Y chromosome could serve, therefore, as marker for karyotyping of tumor cells (in RBT157). RBT323 passage 7 and RBT157 passage 4 were studied. Both tumors had a near-diploid chromosome number with numerical and structural aberrations. Representative karyotypes are depicted in Fig. 4. The two independently arising tumors had loss of chromosomes 5 and 20 in common. Chromosome 12 was absent only in RBT157. RBT323 exhibited additional loss of chromosomes 7, 8, 10, 14 and 19. Interestingly, chromosome 8 was also involved in a structural abnormality (8p+). No common structural changes were found.

Discussion

In an autopsy series on 300 ACI rats we found five TCC. Two appeared to be serially transplantable (RBT323 and RBT157). Upon transplantation, tumor biological characteristics changed. The tumor doubling time of the RBT323 tumor decreased from 13 to 3.5 days. The doubling time of the RBT157 tumor was stable at 8 days. The most important tumor biological change was the acquisition of metastatic ability. The RBT323 tumor changed in the fifth passage from low metastatic ability – less than 5% of the animals had metastases – to high metastatic ability – more than 90% of the animals showed lung metastases and occasionally lymph node metastases. This suggested a hematogenic pathway. The metastatic ability of the RBT157 tumor changed in the fourth passage from low to intermediate; about 50% of the animals had microscopic lung metastases.

Light microscopy showed a grade II TCC in the initial passages of the RBT323 tumor, which changed in the third passage to grade III. The histological findings, therefore, did not correlate with the phenotypic changes. The discrepancy was even more noticeable in the case of the RBT157 tumor, which histologically remained essentially unchanged, despite its progression to a moderate metastatic phenotype.

Electron microscopically, superficial cells of the mammalian bladder are typically lined by an asymmetrical unit membrane (AUM). Within these cells numerous oblong elliptical vesicles derived from the Golgi area are found [17, 19, 41]. The fact that we could demonstrate these vesicles, although in an aberrant form, stresses the urothelial origin of the bladder tumors. Some cells in the intermediate layer contain electron-dense membrane-bound granules resembling (neuro)-endocrine granules; these cells have also been described in human TCC [7, 15].

Intermediate filament proteins are expressed in tissue-type-specific fashion and are therefore useful in tumor diagnosis [27]. RBT323 and RBT157 express cytokeratins and can therefore be considered of epithelial origin. So far

20 different cytokeratins have been characterized, and their expression pattern is characteristic for the differentiation stage [4, 23, 25]. Transitional cell epithelium typically shows expression of cytokeratins 5, 7, 8, 18 and 19 [24]. The synthesis of cytokeratins is usually maintained during malignant transformation and can, therefore, be exploited in specific tumor diagnosis. With the panel of monoclonal antibodies used in this study we could not detect changes in the ratio of RGE 53-positive cells to total cytokeratin positive cells: enrichment of RGE 53-positive cells, which marks high-grade human TCC, was not evident in this model system [31]. The differences in the number of RGE-53 positive cells among the passages of the RBT323 tumor could be explained by the phenomenon of epitope unmasking or epitope formation [36]. These changes in cytokeratin 18 expression might be overcome by the use of different anticytokeratin 18 monoclonal antibodies [36]. Furthermore, it should be noted that RKSE 60, recognizing cytokeratin 10, which is found in keratinizing epithelium, was negative. This indicates that no SCC components are present in these tumors [13].

The tumor lines here described are of urothelial origin and the progression to a metastatic phenotype is not associated with gross changes, although in the case of RBT323 the histology progressed from grade II to grade III. In order to obtain more insight in the genetic changes that occurred in these tumors, karyotype analyses were performed on the tumor lines once they had acquired a more stable phenotype (RBT323 passage 7, RBT157 passage 4). Comparative analyses showed that both lines were peridiploid and that in these independently arising tumor lines chromosomes 5 and 20 were lost. The loss of chromosome 5 is especially interesting, since human chromosome 9 is syntenic to the major portion of rat chromosome 5 [21]. In human TCC loss of chromosome 9 is frequent [14, 26, 44] and is considered an early event, since the chromosome is already lost in most superficial tumors. This underlines the similarity between our model and human superficial TCC. The more aggressive subline RBT323 has considerably more cytogenetic changes (both structural alterations and loss of chromosomal segments). Due to the fact that the comparative genomic map of man and rat is rather restricted [21], it is difficult to speculate about the genes potentially relevant to these cytogenetic changes. Interesting locations are 1q and 8p, since in the aggressive line RBT323 both copies are truncated (two translocations for 1q, loss of one copy and a translocation for 8p). Of the additional losses, 19 is of particular interest since it is syntenic to human chromosome 16q. Loss of heterozygosity on 16q22-ter is frequently found in several human cancers [6]. Thus comparative cytogenetic analysis can provide further clues to loci relevant to the development of bladder cancer.

Considering the phenotypical characteristics, the cytogenetic changes and the initial only slightly aggressive biological potential of the tumors we believe that these lines can be used to study the progression of human superficial bladder cancer to invasive status. It is especially interesting that the tumor lines during serial passaging progress to a more aggressive status at different rates (RBT323 fast vs RBT157 slow). Our working hypothesis is that the initial passages can be used to study the progression of the tumors and the molecular changes that are occurring during progression. For this purpose a batch of viably frozen tumor pieces of the first transplant passages are preserved.

Thus, this model may be helpful for the identification of specific molecular steps associated with the progression of bladder cancer.

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