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Features of Squamous and Adenocarcinoma in the Same Cell in a Xenografted Human Transitional Cell Carcinoma: Evidence of a Common Histogenesis?*

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Summary. Ultrastructural features of squamous differentiation have been found in adenocarcinomatous cells in a xenografted line (UCRU-BL-17) established in nude mice from a primary human bladder transitional cell carcinoma (grade III, stage T4) with a tetraploid DNA component. The line has been characterized by light and electron microscopy, flow cytometry and immunocytochemistry. The initial xenograft showed predominantly adenocarcinomatous differentiation with mucin secretion, whilst the subsequent passages also contained cells showing squamous differentiation. A xenograft subline established from a cell culture of the initial xenograft shows the emergence of a population of cells with near triploid DNA, which are less differentiated, grow more quickly, show decreased expression of carcinoembryonic antigen, and a change in the distribution of staining with peanut lectin from cell surface to cytoplasm. These lines offer an unusual opportunity to study the histogenetic relationships between the histological subtypes of bladder cancer.

Key words: TCC – Lineage heterogeneity – Xenograft – Squamous bladder carcinoma – Adenocarcinomatous differentation

Introduction

The utility of the xenograft as a model of human malignancy has been documented for many tumour types [3, 5, 10, 14]. We have used the xenograft model to study functional heterogeneity of urothelial transitional cell carcinoma xenograft [12]. However, these data did not clarify the developmental relationship between the histological subtypes of bladder cancer: Specificially, whether transitional cell carcinoma (TCC), adenocarcinoma and squamous carcinoma evolve from a common progenitor or stem cell, or have independent origins. We report the characterisation of a xenograft line, UCRU-BL-17, derived from a TCC with elements of adenocarcinomatous differentiation, in an attempt to explore this subject.

Materials and Methods

Mice

Male BALB/c nu/nu ("nude") mice, aged 8–12 weeks were obtained from the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia, and were housed in standard cages fitted with filter tops and handled in laminar flow hoods. They were fed irradiated standard mouse diet and sterile acidified water ad libitum.

Patient Details

A 69 year old female presented with a grade III, stage T4b transitional cell carcinoma of the bladder, with elements of adenocarcinomatous differentiation. She received intravenous cisplatin and radiotherapy but sustained only a transient remission and died 43 months after presentation. Autopsy was not performed.

Xenografts

Tumour fragments from the TCC biopsy measuring approximately 2 mm^3 were inoculated s.c. through small incisions bilaterally in the scapular region, and serially passaged as described previously [12]. Tumour cell suspensions from cell culture were inoculated s.c. at 10^6/0.1 ml.

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Light and Electron Microscopy

Specimens for light microscopy from the original tumour and xenografted passages were fixed in buffered 10% formalin, were embedded in paraffin and stained with haematoxylin and eosin, periodic-acid Schiff (P.A.S.) plus diastase, mucicarmine and alcian blue for mucin. Tissue blocks of xenograft specimens of approximately 1 mm^3 were fixed in cacodylate-buffered 2.5% glutaraldehyde and processed for electron microscopy as previously described [12].

DNA Flow Cytometry

The cellular DNA content of specimens of the original tissue and of xenografts was measured by flow cytometry following staining with propidium iodide as previously described [12]. An internal DNA standard of chicken erythrocytes at a concentration of 3×10^5/ml was used. Analyses were performed using a Cytofluorograf System 50H cell sorter (Ortho Instruments, Westwood, Mass) with an argon-ion laser excitation source at 488 nm.

Immunocytochemistry

Immunoperoxidase staining for carcinoembryonic antigen (CEA), beta-subunit human chorionic gonadotrophin (B-HCG), peanut lectin (PNA) and for keratin was carried out using the unlabelled antibody enzyme method (PAP) on dewaxed paraffin sections [12]. Antibodies to CEA and B-HCG and conjugates were obtained from Dakopatts, Copenhagen, Denmark; PNA and rabbit anti-PNA were obtained from Sigma Chemical Co., St. Louis, Mo. The positive controls for testing included normal human colon (CEA), human trophoblast (B-HCG), Hodgkin's lymphoma (PNA) and human skin (keratin). An irrelevant first antibody was used for negative controls.

Measurement of Prostatic Acid Phosphatase

Fluid was aspirated from larger cystic xenografts and centrifuged at 300G to obtain a cell-free supernatant. This was tested for the presence of human prostatic acid phosphatase by radioimmunoassay, kindly performed by Dr. Peter Stewart, Department of Biochemistry, Royal Prince Alfred Hospital, using a standard technique (Abbott Prostatic-Acid Phosphatase Enzyme Immunoassay Kit, Abbott, Laboratories, Diagnostic Division, North Chicago).

Results

Establishment and Characterisation of Xenografts

The initial implanted human biopsy grew in nude mice after 7 months and was both serially passaged in mice (UCRU-BL-17) and established as a continuous cell line in vitro at this point [13]. The cell line was reinoculated into nude mice after 20 passages in tissue culture, and the resulting tumour further transplanted to produce a second xenograft line.

Some differences were observed between these two lines. The xenograft line established from the patient biopsy, UCRU-BL-17, grew slowly with a long lag period of approximately 5–6 months, which persisted on serial passage (Fig. 1) and was associated with severe cachexia in the recipients. This line is currently in passage 3. The xenografted line established from tissue cultured cells, UCRU-BL-17CL (currently in passage 7), grew more rapidly, without a detectable lag period (Fig. 1), and did not cause cachexia. Initially, tumours of both lines grew as solid subcutaneous masses of uniform consistency, with no macroscopic evidence of invasion or metastases. However, at passage 2, UCRU-BL-17CL tumours of large size suddenly developed into fluid filled cysts which grew rapidly over 3–4 days. Cysts have consistently formed on serial passage of this line. Fluid recovered from cysts at sequential passages contained viable cells and human prostatic acid phosphatase. These findings have been verified in samples from both male and female tumour-bearing mice. Karyotyping confirmed the presence of human, not murine, chromosomes in these xenografted tumours. The karyotype of the tissue cultured line derived from UCRU-BL-17, is shown elsewhere [13]. There were several consistent markers including an inverted 1p, an isochromosome 5p and an 11,13 translocation.

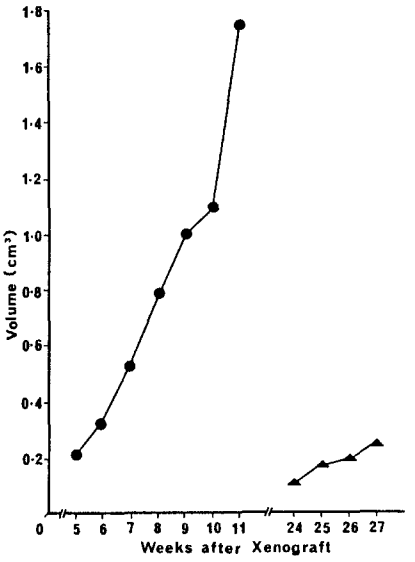

Fig. 1. Growth of Xenograft lines in nude mice. ▲–▲, growth of line established from original tumour, UCRU-BL-17, passage 2 (X2). ●–●, growth of line established after tissue culture, UCRU-BL-17CL, passage 2 (X'2)

Histology and Immunocytochemistry of Xenografts

The morphology of the tumours is compared in Table 1 and Fig. 2. The patient's tumour consisted of a mixture of TCC with some squamous and adenocarcinomatous differentiation. All three cell types were consistently seen in xenograft passages. However, the initial xenograft showed predominantly adenocarcinomatous elements with mucin production whilst some cells showing squamous differentiation were present in later passages. The tumours resulting from

Table 1. DNA flow cytometry and morphology of UCRU-BL-17

Specimen	FCM[a]		Morphology[b]	Mucin Production			E.M.
	Ploidy	%S		Alcian blue	P.A.S. + D	Muci-carmine	
Original tumor	2n (90)	19	TCC, gd 2–3, sq, gl.	++	++	++	Not done
Xenografts[c]							
X0	2n (90) 4n (10)	23	TCC, gd 2–3, gl.	+++	+++	+++	Mainly gl.
X1	2n (90) 4n (10)	29	TCC, gd 2–3, sq, gl.	+++	+++	+++	Mainly gl.
X2	2n (81) 4n (8)	11	TCC, gd 3, sq, gl.	+++	+++	+++	Not done
X'0	2n (77) 4n (23)	15 24	TCC, gd 3, mainly gl.	++	++	++	Mainly gl.
X'1	2n (28) 3.5n (48)	17	TCC, gd 3, less diff.	+	–	+	gl (+ sq)
X'2 solid	2n (81) 3.6n (19)	d	TCC, gd 3, less diff.	++	++	++	Not done
fluid	2n (79) 3.6n (21)	d					

Proportion of cells showing positive staining for mucin: + = less than 1/3 of cells; ++ = 1/3 to 2/3 of cells; +++ = > 2/3 of cells

[a] FCM: DNA Flow Cytometry; Ploidy: numbers in parentheses are percentages of total G0/G1 cells; %S: percentages of cells in the synthetic phase

[b] Morphology: sq = squamous; gl = glandular; TCC = transitional cell carcinoma

[c] Xenografts: X = derived from original tumor implanted in nude mice. Number represents passage number. X0 = intitial take; X' = derived from cell line, UCR-BL-17CL, inoculated into nude mice

[d] Unable to be calculated because of the distribution of cells

Table 2. Immunocytochemistry of UCRU-BL-17

Specimen	Presence of immunoperoxidase staining for:				
	EMA	CEA	PNA[a]	B-HCG	Keratin
Original tumor	not done	++	+	–	not done
Xenografts[b]					
X0	not done	+++	+++ (s)	–	not done
X1	not done	+++	+++ (s)	–	not done
X2	+ (some cells)	+ (few)	+++ (cy & s)	+ (few)	+++
X'0	+++	+ (few)	+++ (cy)	–	+++
X'1	+++	–	+++ (cy)	–	+++
X'2 solid	+ (some cells)	+ (few)	+++ (cy)	–	+++

Intensity of staining: + = weakly positive; ++ = of moderate intensity; +++ = strongly positive

[a] PNA staining: s = surface; cy = cytoplasmic

[b] Xenografts: X = derived from original tumor implanted in nude mice. Number represents passage number. X0 = initial take; X' = derived from cell line, UCR-BL-17CL, inoculated into nude mice

inoculation of tissue cultured cells (UCRU-BL-17CL) showed more cellular pleomorphism than those of UCRU-BL-17, but were consistent with a TCC, grade III. In particular, adenocarcinoma cells appeared more bizarre and less well differentiated. In one instance, these cells were seen to invade the perineural space (Fig. 2C).

The results of immunocytochemical staining for CEA, B-HCG, PNA and keratin are summarized in Table 2. The original human tumour and its xenografts (UCRU-BL-17) showed a high percentage of cells staining positively for CEA, which tended to correlate with adenocarcinomatous differentiation. These tumours also showed intense binding

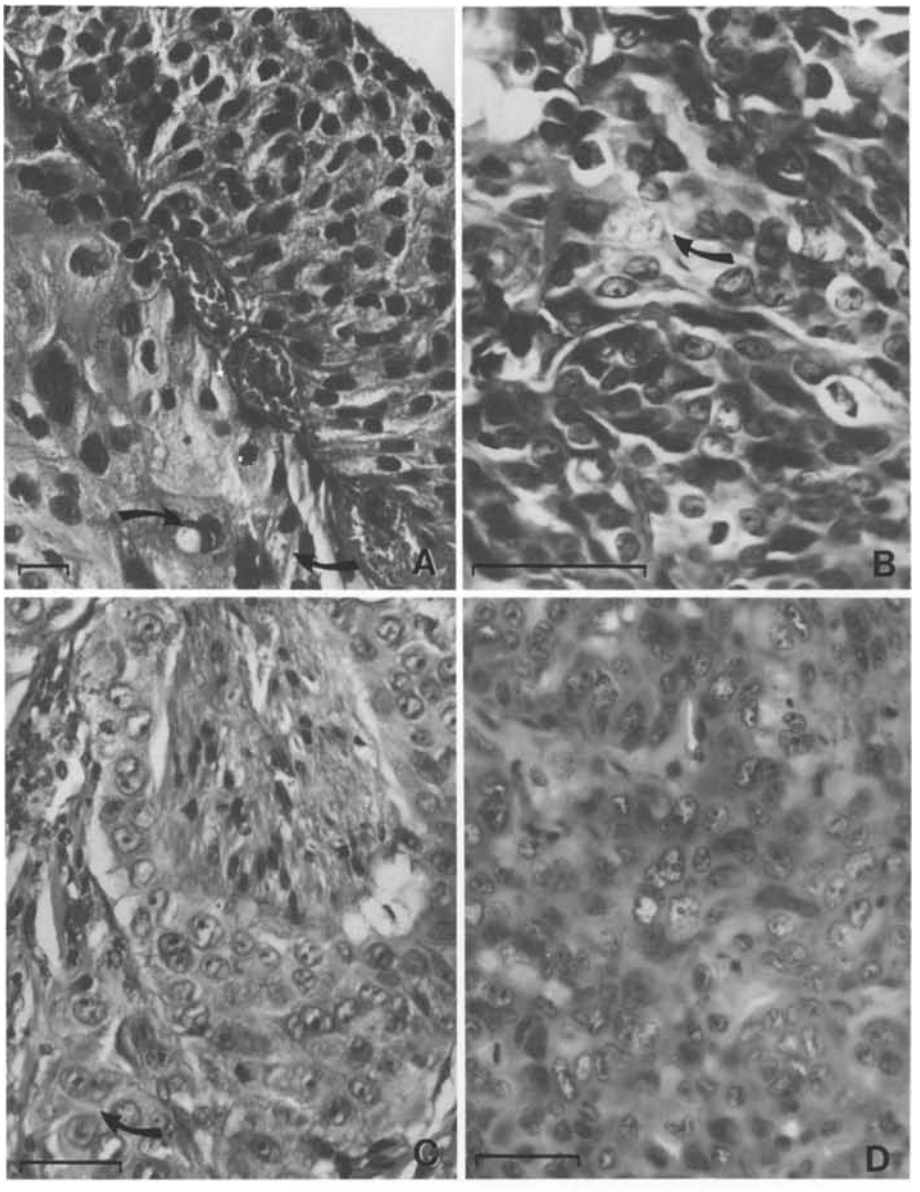

Fig. 2A–D. Bars represent 25 μM in each case. **A** transitional cell carcinoma with squamous and adenocarcinomatous differentiation (*arrows*) (original tumour). Light micrograph, Haematoxylin and eosin, x280. **B** area of predominantly adenocarcinoatous differentiation (*arrow*) (Xenograft, X0 established from original tumour). Light micrograph, haematoxylin and eosin, x860. **C** perineural invasion and squamous differentiation (*arrow*) (Xenograft, X′0, established from tissue cultured line, UCRU-BL-17CL). Light micrograph, haematoxylin and eosin, x560. **D** poorly differentiated cells with increased nuclear pleomorphism (Xenograft, Passage one, X′1, established from tissue cultured line, UCRU-BL-17CL). Light micrograph, haematoxylin and eosin, x560

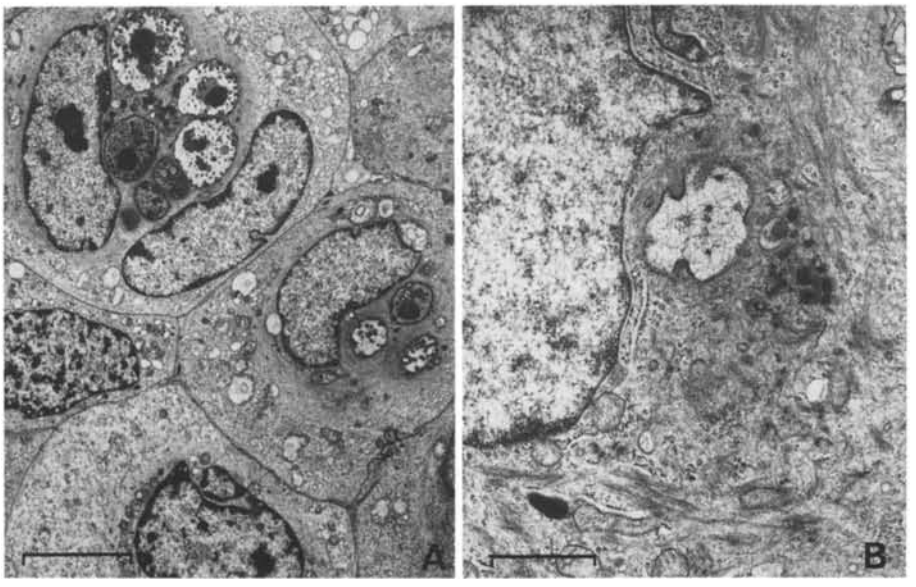

Fig. 3. A adenocarcinomatous differentiation in xenograft, UCRU-BL-17. Electron micrograph, x3,500 (bar = 0.7 μM). **B** Single cell showing both adenocarcinomatous and squamous differentiation within the one cell (Xenograft, UCRU-BL-17). Electron micrograph, x13,200 (bar = 2.8 μM)

to PNA with predominantly surface distribution in the first passages, and additional cytoplasmic staining in later passages. In contrast, the xenografted tumours derived from tissue cultured cells showed weaker CEA staining, confined to a few cells, and intense cytoplasmic PNA staining. Immunocytochemical staining for prostatic acid phosphatase was negative in the original tumour and in the xenografts.

Ultrastructure of Xenografted Tissue

At the ultrastructural level, the xenografts formed sheets and trabeculae of cells or smaller nests separated by collagenous connective tissue bands. At the periphery, tumour cells were sometimes surrounded by a "capsule" of compressed collagen, but more often infiltrated fibroadipose tissue. A basal lamina was rarely in evidence. In all samples, a mixture of cell types was present, with the most striking being those showing adenocarcinomatous differentiation (Table 1, Fig. 2): these occasionally formed acini limited by junction complexes, but more often were characterised by the presence of multiple intracellular lumina lined by microvilli with a prominent glycocalyx, secretory material in the lumen and occasional mucin droplets in the surrounding cytoplasm. Typical well developed squamous cells with interlacing dense tonofilament bundles in their cytoplasm were uncommon, but there were scattered cells containing more loosely arrayed filaments and forming intracellular bridges joined by small tonofilament-desmosome complexes.

The most interesting finding was that some cells showing squamous differentiation also contained intracellular lumina with evidence of mucin production. This implied the presence of both squamous and glandular differentiation within the one cell (Fig. 3B). The remaining cells were either of intermediary transitional type or were completely undifferentiated.

DNA Flow Cytometry

The most striking feature of the DNA content of the human tumour was the high percentage of cells in the synthetic phase (S) of the cell cycle, and this was maintained in xenografts in nude mice (Table 1). The donor tumour, which was predominantly diploid, showed a tetraploid component, which was also expressed in the xenografted material (UCRU-BL-17). Xenografts established from the tissue cultured cells also had a near triploid component.

Discussion

It is not clear whether urothelial TCC and the other variants of bladder neoplasia (adenocarcinoma and squamous carcinoma) represent different diseases or whether they arise from a common stem cell. The natural history, aetiology and sensitivity to treatment of these subtypes is substantially different [1, 6, 8]. However, TCC, the commonest form of bladder cancer, may coexist with elements of adenocarcinomatous and squamous differentiation. In the rat, exposure to the carcinogen, N-butyl-N-4-hydroxybutyl-nitrosamine (BBN), is associated with the ultimate development of synchronous squamous and transitional cell carcinomas but not adenocarcinoma of the bladder [4, 7].

The work described in this paper demonstrates the presence of ultrastructural features of both squamous and adenocarcinomatous differentiation within the one cell in a xenografted line derived from a human TCC. In a parallel study of a cell line derived from the same tumor, keratin ringing has been noted in mucin containing cells in vitro [13]. These observations support the concept of a common stem cell of origin of cells showing squamous or adenocarcinomatous features. Lineage heterogeneity has been demonstrated in cases of acute leukaemia, with evidence of leukaemic blasts concurrently expressing both lymphoid and myeloid features at diagnosis, or a lineage switch, characterized by a sequential shift from lymphoid to myeloid phenotypic expression, or the reverse [15]. A tissue culture cell line derived from the second xenograft passage of UCRU-BL-17 has been cloned by limit dilution (Brown et al. in preparation), and these subclones will be used to further explore the relationship between bladder carcinomas showing squamous, transitional or adenocarcinomatous features.

The significance of the development of a near-triploid DNA content after growth of the tumour cells in vitro is not clear. This could represent an outgrowth of a less dominant population of cells present in the original tumour (for example, due to the altered environment), or possibly a natural evolution of the tumour cells themselves. The triploid DNA content is associated with histological dedifferentiation of the tumour, a faster growth rate in vivo, reduced staining for CEA and a change in the distribution of binding to PNA. The failure to cause cachexia in tumour-bearing mice is currently being studied in our laboratory.

The detection of human prostatic acid phosphatase in fluid aspirated from cystic areas of the xenografts is of interest, especially as this antigen was not detected on routine immunocytochemical staining of "donor" or xenograft tissues. The demonstration of high concentrations of tumour antigens in such cystic xenograft fluid has previously been reported for germ cell tumours [9] and probably reflects the high ratios of tumour mass: mouse weight and tumour mass: cyst fluid volume. Reid et al. [11] previously showed that a xenografted bladder tumour (R198) derived from a male patient produced prostatic acid phosphatase in male but not in female recipients. The differentiation features expressed by this tumour appeared to be sex dependent. In contrast, the line, UCRU-BL-17CL, was derived from a female patient, and xenografts were found to show similar histological features, and to produce prostatic acid phosphatase in both male and female nude mice. As the prostate and bladder are intimately involved anatomically and embryologically, and tumours of each organ have been

reported to coexist in the one patient [2], the xenografts, R198 and UCRU-BL-17, may imply a common histogenesis of some tumours of these organs.

We believe that the xenografted lines described here offer an unusual opportunity to study the possible mechanisms by which histological subtypes of bladder cancer may develop from a common stem cell. We are currently using the lines to study the activation and expression of oncogenes, and differences in membrane proteins in cells expressing differentiation features of the different histological subtypes of bladder cancer.

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