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The Growth of Human Bladder and Kidney Cancers as Xenografts in Nude Mice and Rats

P. N. Matthews*, A. G. Grant and J. Hermon-Taylor

Department of Surgery, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, Great Britain

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Summary. Primary human kidney and bladder tumours were implanted into both nude rats (rnu/rnu) and nude mice (nu/nu). 40% of bladder tumours and 27% of kidney tumours were established as xenografts. Histological sections of primary tumour and xenografts confirmed their similarities. Two patients with renal tissues were hypercalcaemic prior to nephrectomy, and animals in which these tumours grew as xenografts also became hypercalcaemic. Cell lines were established in tissue culture from two of the kidney tumour xenografts, and chromosome studies have confirmed their human karyotype.

Key words: Kidney and bladder tumour xenografts, Nude mice and rats, Hypercalcaemia.

Introduction

Bladder cancer now causes over 4,000 deaths per year in England and Wales and this figure has been steadily increasing over the last fifty years [13]. More than half of those who present with bladder cancer will have T1 tumours confined to the mucosa [17] and the prognosis of these patients is generally good with 5 year survival rates of about 70% [17, 2]. Although most early bladder tumours can be controlled by regular endoscopic procedures, nearly one third of these tumours will progress and become invasive with the result that these patients have a poor prognosis often needing major surgery, radiotherapy or chemotherapy. There is at present no reliable method of predicting which tumours will progress and how they will respond to further therapy.

Carcinoma of the kidney is also increasing in incidence [14] and the rather poor prognosis of patients with this tumour is related to their late presentation and the poor response to any treatment other than surgery.

Since Rygaard and Povlsen [19] first demonstrated that human colonic tumours could be grown as xenografts in "nude" athymic mice there have been reports of many human tumour xenografts in the nude mouse [9] and more recently the nude athymic rat [7, 6]. However, there are still very few reports describing the establishment of either kidney or bladder tumour xenografts [21, 24, 12].

This present work was undertaken to investigate whether the nude mouse and the nude rat could act as suitable in vivo models for the further study of the biology of kidney and bladder tumours.

Materials and Methods

Animals

Outbred congenitally athymic "nude" mice (nu/nu) were obtained from the Imperial Cancer Research Fund Laboratories (Mill Hill, UK) and housed in germ-free negative-pressure plastic isolators. Outbred congenitally athymic "nude" rats (rnu/rnu) were obtained from Olac Ltd. (Bicester) and housed in filter boxes. Operative procedures on mice were performed in the isolators and on the rats in a Hepaire flow cabinet. The rats were anaesthetised prior to operation using Halothane and nitrous oxide supplied through a standard Boyles anaesthetic machine.

Tumour Collection and Inoculation

All bladder specimens were obtained from patients undergoing transurethral resection of their tumours. The tumours were resected using the cutting current to minimise charring and glycine was used as the irrigating fluid rather than water which causes cell lysis. Bladder tumours were classified according to the UICC TNM system (1978) [23] and graded 1–3 with increasingly poorly differentiated histology. Six of the kidney tumours had been embolised prior to surgery in an attempt to necrose the tumour in situ. Following radical nephrectomy the specimens were sectioned in theatre and the most viable-looking tumour was reserved for xenografting. The tumour tissue was immediately placed in Hams F12 culture medium supple-

* P. N. Matthews is now a lecturer in Urology at the Royal Marsden Hospital, London

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Table 1. Growth characteristics of human bladder tumour xenografts in nude mice (nu/nu) and rats (rnu/rnu)

Patient	Tumour Stage	Tumour Grade	Growth in nude rats		Growth in nude mice		Tissue Culture (No. of passages)	
			Number growing/number implanted	Lag Phase (weeks)	Number growing/number implanted	Lag Phase (weeks)	1° Explant	Xenograft
CB	pTa	GI	0	–	0/4	–	–	–
GR	pT1	GII	0/2	–	0/5	–	–	–
FM	pTa	GI	0/2	–	0/5	–	0	–
TT	pT1	GII	0/2	–	0/4	–	–	–
KN	pT2	GI	1/1	20	2/3	10	–	3
DD	pT1s	GI	0	–	0/4	–	–	–
LJ	pT2	GIII	0	–	1/2	30	–	2
OO	pT1	GII	0	–	0/2	–	–	–
RL	pT1	GI	0	–	0/4	–	–	–
FG	pTa	GII	0	–	1/1	22	–	2
FF	pTa	GI	0	–	0/2	–	–	–
AO	pT1	GII	0	–	0/1	–	–	–
RH	pTa	GI	0/3	–	2/3	24	1	1
PE	pT2	GI	0	–	2/4	24	–	–
AF	pT1	GII	0	–	2/2	10	–	2
AS	pT2	GII	0	–	0/4	–	–	–
RB	pTa	GI	2/2	18	0/1	–	2	–
GM	pT1	GII	0	–	0/1	–	–	–

mented with 10% newborn bovine serum, glutamine 5 g/l, gentamicin 50 mg/l, penicillin-G 200,000 iu/l and streptomycin 200 mg/l (Flow Laboratories, UK), transferred to the isolator and minced finely with sterile scissors. The resulting tumour mush was inoculated into a subcutaneous epigastric site using a 16-guage transplant needle as previously described by Grant et al. [10]. The volume of tumour inoculated was 0.4 ml to a single site on each mouse and 1.0 ml to each rat.

Observation of Tumour Growth

Animals were observed weekly for signs of tumour growth. When tumours started growing they were measured weekly using calipers, and the result was expressed as the product of two perpendicular diameters. If no tumour had appeared by 35 weeks the animals were sacrificed. Mice were sacrificed and tumours excised when tumour size was greater than 3 cm^2 while in rats the tumours grew to 5 cm^2, after which they were excised under general anaesthetic and the animals allowed to recover. A small piece of excised tumour was fixed in 10% formalin for histological examination, another piece was placed in supplemented Ham's F12 medium for tissue culture and the remainder was inoculated into further animals.

Tissue Culture

Primary or xenograft tumour was finely minced with crossed scalpels and cultured in Falcon flasks using supplemented Ham's F12 medium. Growth was observed daily and when cells became confluent they were passaged after harvesting with 0.02% EDTA in calcium and magnesium free Earle's (Flow Laboratories). Human tumour origin was confirmed by chromosome analysis [20].

Established cell lines were grown in supplemented Ham's F12 medium, and following harvesting were suspended in phosphate-buffered saline (1×10^7 cells per 0.2 ml) and inoculated subcutaneously into nude mice. Bladder tumour cell lines; J82 [16], TCC Sup [15] and T24 [4] were kindly supplied by Carol O'Toole. RT4 [18] and EJ [11] were supplied by Dr. L. M. Franks, Imperial Cancer Research Fund. A kidney tumour cell line (Caki-1) was kindly supplied by Dr. J. Fogh (Sloane Kettering Institute, New York).

Calcium Studies

Total plasma calcium levels in patients were measured using a standard Technicon autoanalyser and corrected for plasma proteins. Animal plasma calcium was measured on a 40 μl sample using the micro-method of Cheung [5].

Results

Bladder Tumours

18 transitional cell carcinomas of the bladder were transplanted into 51 mice and 12 rats. All tumours were implanted into mice and if enough tumour tissue remained, into rats as well. Table 1 shows the results and correlates tumour stage and grade with the ability of the tumour to grow as a xenograft. In all animals the nodule that was originally present following inoculation disappeared within 3 weeks. The lag phase before growth ranged from 10 to 30 weeks in the 10 nude mice and was about 20 weeks in the three rats. Tumour growth was extremely slow, often taking a year to reach a transplantable size (3 cm^2) in mice, while in rats none of the tumours had reached a transplantable size (5 cm^2) after 1 year. Of the 18 primary tumours implanted, seven (39%) showed successful xenograft growth. Four of these tumours in mice have passaged successfully; the slow growth rate is maintained in 3 while the growth rate of the fourth (PE) has substantially increased.

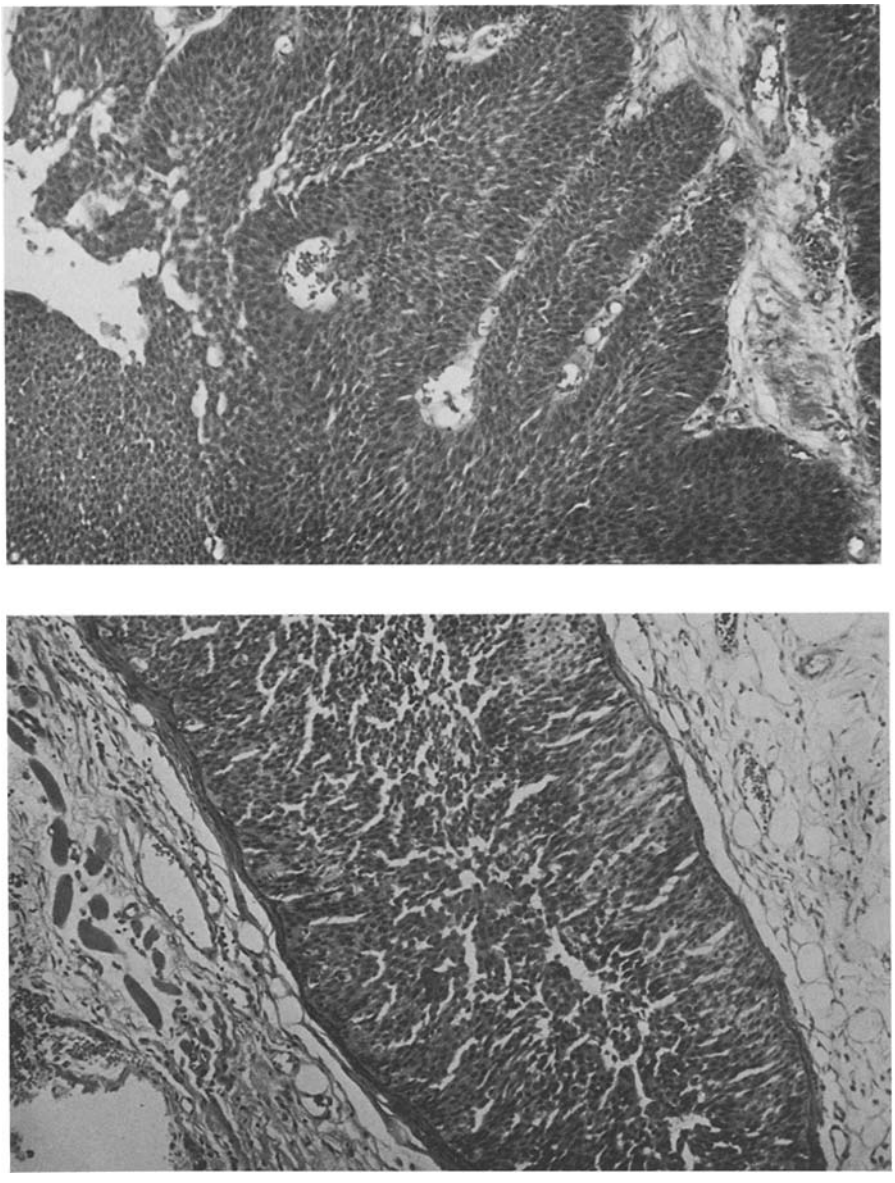


Fig. 1. Histological section (x 23) of a well-differentiated bladder cancer (KN); (a) primary tumour (b) nude mouse xenograft

Tumours from three patients (AF, AS, FG) formed fluid containing cysts at the inoculation site and on sectioning, the whole of the cyst wall was found to be composed of tumour. The histological features of the xenograft tumours closely resembled the primary tumour from which it was grown in all cases; an example of this is shown in Fig. 1.

Renal Tumours

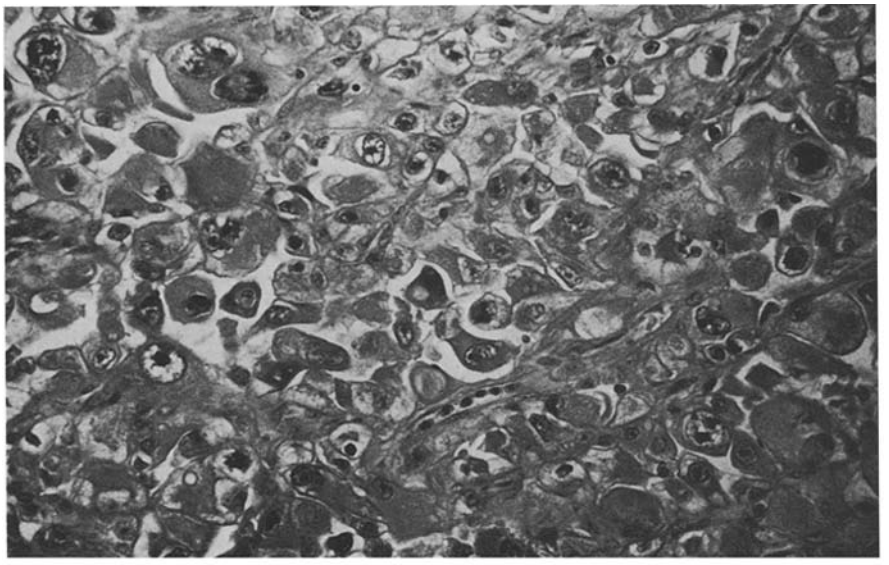
11 carcinomas of the kidney were transplanted into 51 mice and 27 rats and the results are shown in Table 2. The three tumours which grew as xenografts were all poorly differentiated with a predominantly granular cell type.

There were several differences between the growth characteristics of kidney and bladder tumour xenografts. The initial subcutaneous nodule following renal tumour inoculation never disappeared in animals in which succesful growth subsequently occurred. There was a lag phase of approximately 6 weeks before tumour growth in rats and mice, and the tumours grew more quickly and reached a transplantable size after about 12 weeks in mice and between 30 and 70 weeks in rats. The histological appearances of the xenograft again closely resembled that of the primary tumour (Fig. 2). Equal volumes of primary tumour (CG) implanted simultaneously into two rats demonstrated markedly different growth rates (Fig. 3). The rapidly growing tumour (Rat A) regrew at the same rate following partial or macroscopically "complete" excision.

Two of the patients (TM, AW) with kidney tumours were hypercalcaemic prior to surgery, with corrected total plasma calcium concentrations of greater than 3.5 mmols/l (normal 2.2–2.6 mmols/l); these calcium levels returned to normal within 2 days of nephrectomy. All animals bearing these tumours became hypercalcaemic with total plasma calcium levels of 3.5–4.2 mmols/l. Mean plasma calcium

Table 2. Growth characteristics of human kidney tumour xenografts in nude mice and rats

Patient	Pre-operative Embolisation	Histology Cell Type	Growth in nude rats		Growth in nude mice		Tissue culture (No. of passages)	
			Number growing/number implanted	Lag Phase (weeks)	Number growing/number implanted	Lag Phase (weeks)	1° Explant	Xenograft
CG	Yes	Granular & Spindle	2/3	6	–	–	–	16
TM	Yes	Granular	1/2	6	4/4	5	0	0
LE	Yes	Clear	0/3	–	0/4	–	–	–
HG	Yes	Granular & Clear	0/3	–	0/6	–	–	–
MC	No	Granular	0/3	–	0/6	–	–	–
WS	No	Clear	0/3	–	0/6	–	–	–
KM	Yes	Granular & Clear	0/2	–	0/5	–	0	–
AW	No	Granular & Clear	1/4	6	4/5	5	0	9
AZ	No	Clear	–	–	0/6	–	–	–
MA	Yes	Clear	0/2	–	0/3	–	0	–
JB	Yes	Clear	0/2	–	0/6	–	–	–



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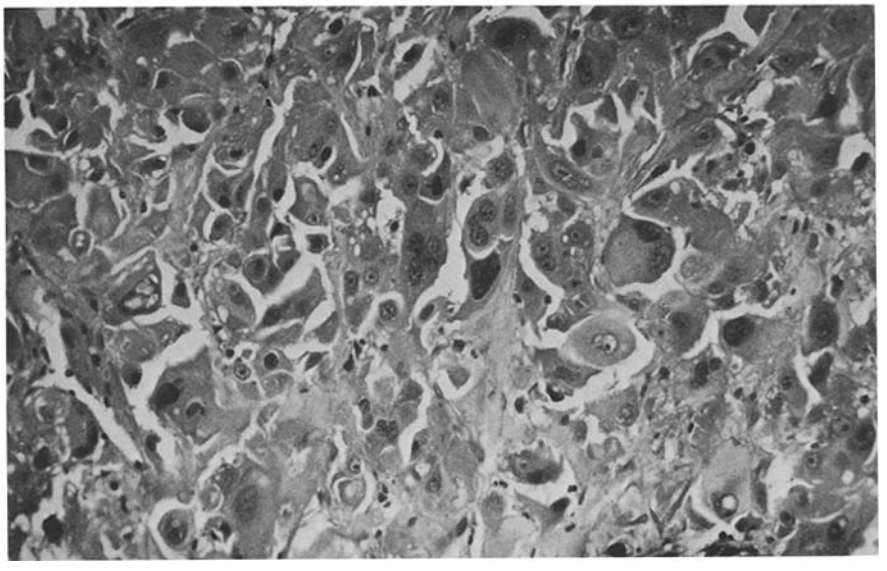

b

Fig. 2. Histological sections (x 46) of a kidney carcinoma (TM); (a) primary tumour, (b) nude rat xenograft

Table 3. Growth characteristics of cultured human tumour cells in nude mice

Cell line	Number of mice inoculated	Number of cells per mouse	Number of mice forming tumours	Lag phase
Bladder tumours				
J82	16	1×10^7	Nil	
Tcc Supp	12	1×10^7	Nil	
T24	8	1×10^7	Nil	
EJ	8	5×10^6	8 (100%)	14 days
RT4	12	5×10^6	9 (75%)	28 days
Kidney tumours				
Caki 1	8	1×10^7	4 (50%)	40 days
GYL	12	5×10^6	8 (60%)	28 days
WIL	10	8×10^6	12 (90%)	28 days

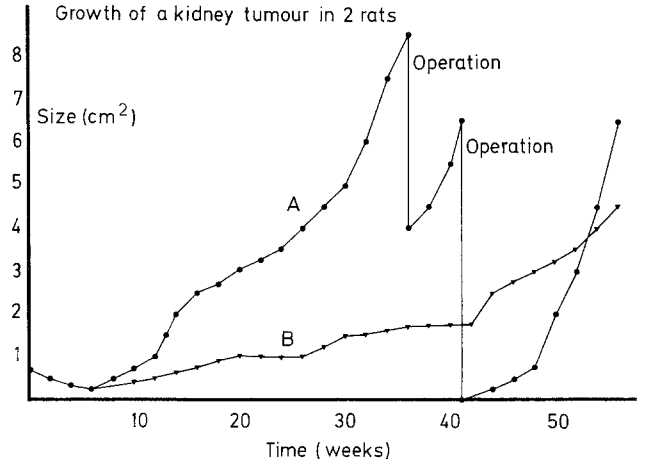

Fig. 3. Growth of a human kidney carcinoma in two nude rats (*A* and *B*). The tumour growing in "Rat *A*" has been partially excised at 36 weeks and "completely" excised at 41 weeks

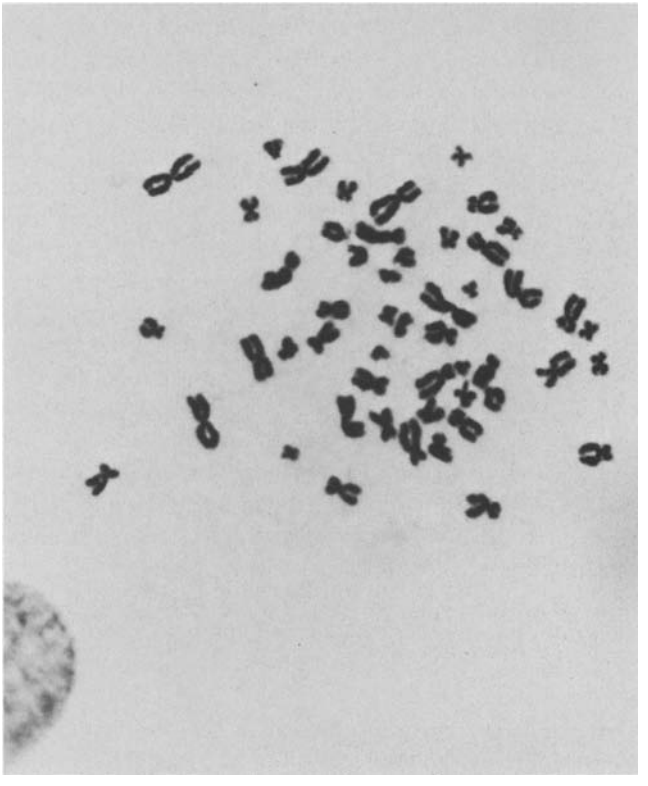

Fig. 4. Chromosome preparation of a renal carcinoma cell line ("GYL") (x 80)

levels in 10 non-tumour bearing nude mice (nu/nu) and six rats (rnu/rnu) were 2.20 ± 0.22 mmols/l and 2.24 ± 0.22 mmols/l respectively. Plasma calcium levels in animals bearing other tumours were in the normal range.

Tissue Culture

Attempts were made to culture the bladder and renal cancers in vitro both from the primary explants and from the xenografts (Tables 1 and 2). Confluent layers of epithelial cells were established initially for most bladder tumour explants and xenografts, but could not be maintained for more than two or three passages.

We were unable to establish any of the primary kidney tumour explants in tissue culture, but tumour samples obtained from two of the three xenografts grew well (CG & AW). Both these tumour cell lines have now been passaged more than 25 times in culture and have also been re-established in culture from later xenograft passages.

Table 3 shows the tumourgenicity of the cell lines in nude mice. Three of the established bladder tumour cell lines (J82, TCC & T24) failed to form tumours despite numerous attempts. Our own kidney tumour lines (GYL and WIL) formed tumours in over 60% of mice inoculated. The human karyotype of both of these cell lines has been confirmed by chromosome preparations, one of which is shown in Fig. 4.

Discussion

Heterotransplantation of human cancers to athymic animals provides a useful method of studying the immunobiology of common tumours and their production of hormones and marker substances. A problem with this system as an aid to clinical management however has been the finding that it is usually the poorly differentiated rapidly metastasising tumours that "take" well and that patients with such tumours are often dead before animal experiments can provide useful chemotherapeutic information [1].

In the present study primary kidney and bladder tumours rather than tumour tissue from metastases of patients with advanced disease was taken in the hope that this would lead to a more useful model.

Xenografts were established from 40% of the primary bladder tumours; a "take" rate which agrees well with studies by Sufrin et al. [21] though these authors used apparently more advanced, less well-differentiated tumours. The lag phase before growth of the xenografted bladder tumours in our series was long and variable (10 to 30 weeks) whereas Sufrin et al. [21] described a constant lag phase of 3 weeks in the eight tumours that grew as xenografts. More than half of the xenografts established in our study were from patients with well or moderately well differentiated T1 bladder tumours. It was interesting to note that most of our patients whose tumours grew as xenografts suffered progression of their disease; two patients have died, four patients are being treated for multiple bladder recurrences and only one patient is apparently disease free. Most patients whose tumours did not grow as xenografts are alive and well.

Less than one third of the kidney tumours grew as xenografts and the three that did grow were all poorly differentiated. Two of the kidney xenografts were derived from patients whose renal arteries had been embolised with sterispon 48 h pre-operatively. The subsequent xenograft growth demonstrates the ability of tumour cells to survive ischaemia and questions the benefit of this procedure to these patients.

Renal carcinoma xenografts had a constant lag phase of 6 weeks before growth, showed only minor differences in growth rate in individual mice and were easily passaged. The very marked differences in growth rate of primary tumours transplanted into nude rats may reflect differences of immune responsiveness in individual recipient animals, possibly related to known residual "T" cell activity in the nude rat [3].

There have been relatively few reports on the establishment of human kidney cancers in continuous culture in vivo [8, 12, 22].

In the present study, the establishment of two new tumour cell lines ("GYL" and "WIL") would suggest that the use of xenograft tumour may be a more effective way of deriving cultures of kidney cancer in vitro. In contrast we were unable to establish and maintain any long term bladder cancer cell lines.

In any study of tumours growing out of their natural environment it is very important to confirm that the tumour characterisitcs have not radically changed. Comparisons of histological sections of primaries and xenografts have confirmed their very similar features, even after several passages. The slow growth rate of the bladder tumour xenografts and the tendency of some of them to form cysts suggests that their growth is following the human pattern. One of the most interesting observations confirming the maintenance of the human characteristics concerns the xenografting of the two hypercalcaemia inducing renal cancers. Animals bearing these tumours become cachexic, wasted and markedly hypercalcaemic in a very similar way to the patient with whom the tumour was removed. Further in vivo and in vitro studies on this tumour are now in progress.

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References

1. Bailey M, Jones A, Reghavan D, Selby P, Shorthouse A, Gibbs J, Peckham M (1981) Limitation of human tumour xenografts in individual patient drug-sensitivity testing. Br J Cancer 43:425
2. Barnes RW, Dick AL, Hadley HL, Johnston OL (1977) Survival following transurethral resection of bladder carcinoma. Cancer Res 37:2895–2897
3. Brooks CG, Webb PJ, Robins RA, Robinson G, Baldwin RW, Festing MFW (1980) Studies on the immunology of rnu/rnu "nude" rats with congenital aplasia of the thymus. Eur J Immunol 10:58–65
4. Bubenik J, Baresoua M, Viklicky C, Jaboubkova J, Sainerova H, Donner J (1973) Established cell line of urinary bladder carcinoma (T24) containing tumour-specific antigens. Int J Cancer 11:765–773
5. Cheung P (1980) Methyl alcohol as the stabiliser in a direct colourimetric calcium determination with o-cresolphthalein complexone. Can J Med Technol 42:60–61
6. Davies G, Duke D, Grant AG, Kelly SA, Hermon-Taylor J (1981) Growth of human digestive-tumour xenografts in athymic nude rats. Br J of Cancer 43:53–58
7. Festing MFW, May D, Connors TA, Lovell D, Sparrow S (1978) An athymic mutation in the rat. Nature 274:365–366
8. Fogh J, Trempe G (1975) New Human Tumour Cell Lines. Fogh J (ed) Chapter 5, pp. 115–137. Plenum Press, New York
9. Giovanella BC, Fogh J (1978) Present and future trends in investigations with the nude mouse as a recipient of human tumour transplants. In: Fogh J, Giovanella BC (ed) The nude mouse in experimental and clinical research, Chapt. 14. Academic Press, New York, pp 281–312
10. Grant AG, Duke D, Hermon-Taylor J (1979) Establishment and characterisation of primary human pancreatic carcinoma in continuous cell culture and in nude mice. Br J Cancer 39:143–151
11. Hastings RJ, Franks LM (1981) Chromosome pattern, growth in agar and tumorigenicity in nude mice of 4 human bladder cancer cell lines. Br J Cancer 27:15–21

12. Katsuoka Y, Baba S, Hata M, Tazaki H (1976) Transplantation of human renal cell carcinoma to the nude mice: as an intermediate of in vivo and in vitro studies. J Urol 115:373–376
13. Morrison AA, Cole P (1979) Epidemiology of urologic cancers. In: Javadpour N (ed) Principles and management of urologic cancer, Chapt. 1. Williams and Williams, Baltimore, pp 1–27
14. Muir CS, Nectoux J (1980) Geographical distribution and aetiology of kidney cancer. In: Sufrin G, Beddey SA (ed) Renal Adenocarcinoma. UICC Technical Report Series, vol 49. UICC, Geneva
15. Nayak SK, O'Toole C, Price ZH (1977) A cell line from an anaplastic transitional cell carcinoma of human urinary bladder. Br J Cancer 35:142–151
16. O'Toole C, Price ZH, Ohnuki Y, Unsgaard B (1978) Ultrastructure, karyology and immunology of a cell line originated from a human transitional cell carcinoma. Br J Cancer 38:64–76
17. Payne P (1959) Sex, age, history, tumour type and survival. In: Wallace DM (ed) Tumours of the Bladder, chapt 19. Livingstone, Edinburgh and London, pp 285–306
18. Rigby CC, Franks LM (1970) A human tissue culture cell line from a transitional cell tumour of the urinary bladder: growth, chromosome pattern and ultrastructure. Br J Cancer 24:746–754
19. Rygaard J, Povlsen CO (1969) Heterotransplantation of a human malignant tumour to "nude" mice. Acta Pathol Microbiol. Scand 77:758–760
20. Schwarzacher HG, Wolf U (1974) Methods in Human Cytogenetics, Schwarzacher HG, Wolf U (ed) Springer, Berlin Heidelberg New York
21. Sufrin G, McGarry MP, Sandberg AA, Murphy GP (1979) Heterotransplantation of human transitional cell carcinoma in athymic mice. J Urol 121:159–161
22. Ueda R, Shiku H, Pfreundschutt M, Takahash T, Li LTC, Whitmore WF, Oettgen HF, Old LJ (1979) Cell surface antigens of human renal cancer defined by autologous typing. J Exp Med 150:564–579
23. UICC (International Union Against Cancer) (1978) TNM Classification of malignant tumours, Harmer MH (ed), 3rd edn. UICC, Geneva
24. Wunderli H, Mickey DD, Paulson DF (1979) C-type virus particles in human urogenital tumours after transplantation into nude mice. Br J Cancer 39:35–42

Professor J. Hermon-Taylor
Dept. of Surgery
St. George's Hospital Medical School
Cranmer Terrace
London SW17 ORE
Great Britain