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Growth characteristics of early passage cell lines compared with established TCC bladder lines

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Abstract The growth patterns of established cell lines from bladder transitional cell carcinoma (TCC) were compared with early passage cell lines. The growth of established cell line 5637 was uninhibited in both serum free (basal) and serum containing media. The early passage line (DR) grew only in serum containing medium. This confirms the unreliability of results from biological studies on established (continuous) cell lines.

Key words Growth · Characteristics · Established cell line · Early passage cell line

Established cell lines are invaluable for the study of the biological nature of a tumour at the cellular level and in the evaluation of anticancer drugs. Long-term cell lines established from bladder transitional cell carcinoma (TCC) have been reported [6, 14, 15]. TCC lines have been grown in serum free medium [5, 13], in agar and agar-methyl cellulose containing medium [3, 7]. There is, however, no simple and reproducible in vitro technique for propagating bladder tumours. The mean colony forming efficiency of TCC bladder cells is between 0.018 and 0.031% [4]. Because of the technical difficulties involved in the primary cell culture most anticancer drug studies are done on established cell lines.

An established cell line represents one subpopulation of the original tumour which has adapted to the artificial conditions in culture. Established cell lines also display phenotypic instability during their prolonged in vitro propagation [9]. There are no data available to compare the growth characteristics of early passage lines with those of established cell lines.

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Materials and methods

Early passage cell line

The bladder tumour specimen (1 cm^3) was obtained at the time of endoscopic resection. The tissue was transported in basal medium supplemented with penicillin, streptomycin and amphotericin-B (all from Sigma) and processed within 30–40 min. Part of the tumour was disaggregated mechanically into a single cell suspension using a dissociation kit (Sigma) and the cell viability determined using trypan blue. Another part of the tumour tissue was minced into small pieces (less than 1 mm^3) and several of these placed in 3 ml of medium in each 50 ml culture flask (Costar). The cells were incubated at 36.5° C in humidified atmosphere of 5% CO_2 in air. Constituents of the medium are listed in Table 1. Plating of the cells was seen within 24–48 h and the cell colonies were mostly clustered around the tissue piece. Medium was changed every 2–3 days depending on the change in pH as identified by the colour of the phenol red indicator. Experiments were carried out after 3 or 4 passages of the cells. Cells were plated at a concentration of 3×10^4/ml in each flask. All experiments were done in duplicate.

Established cell line

The TCC cell line 5637 was donated by the Marie Curie Research Institute, Oxted, Surrey. TCC 5637 was established in 1974 and charcterised [8].

Table 1 Medium (200 ml) used for culturing bladder cells

DMEM[a] + Ham's F12 50:50 (V/V)	180 ml
Fetal calf serum	20 ml
l-Glutamine (200 mM)	2 ml
Penicillin (10,000 units/ml) and streptomycin (10 mg/ml)	2 ml
Testosterone (3×10^{-4} M)	20 µl
Hydrocortisone (1 mg/ml in 95% ethyl alcohol)	20 µl
Selenium (30×10^{-4} as sodium selenite)	20 µl

All purchased from Sigma Chem

[a] Dulbecco's modified Eagle's medium
(Catalogue Nos: DMEM with Glucose 1G/L – Sigma D9036; Ham's F12 – Sigma N4888; Fetal calf serum(type II)- Sigma F2138)

Table 2 Combinations of various components with basal medium

1. Basal(B) (DMEM:Ham's F12 + l-glutamine + penicillin + streptomycin + amphotericin-B)
2. Serum(S)
3. Testosterone(T)
4. Hydrocortisone(H)
5. Selenium(Se)
6. Selenium + testosterone
7. Selenium + serum
8. Selenium + hydrocortisone
9. Selenium + testosterone + hydrocortisone
10. Hydrocortisone + selenium + serum
11. Hydrocortisone + testosterone
12. Hydrocortisone + testosterone + serum
13. Hydrocortisone + serum
14. Testosterone + selenium + hydrocortisone
15. Testosterone + serum
16. Serum + testosterone + selenium + hydrocortisone

Characterisation of the early passage cell line DR

DR is derived from a transitional cell carcinoma pT3 G3. After passaging, the cell line was confirmed to be epithelial in origin by cytology, electron microscopy and positive cytokeratin staining.

Cytology

After the third passage the cells were fixed in neutral buffered formaldehyde (10%) and stained by the Papanicolaou's method for morphological study.

Electron microscopy

Ultrastructure studies of early passage cells were carried out by conventional transmission electron microscopy. Fixation of the cells was accomplished in situ by adding 3% phosphate-buffered glutaraldehyde. The cells were then processed by the flat-face embedding method described by Brinkley et al. [2].

Cytokeratin staining

The early passage cells were stained with cytokeratin antibody 18,19 (5D3, AE1/AE3, Biogen).

Cell counting

Aliquots of cells in passage 3 were cultured in basal medium with various supplements (Table 2) in 16 different numbered culture flasks and growth curves determined. The cell count at the initial plating was done with a Neubauer's haemocytometer. For the growth curve determination a graticule mounted in the eyepiece of the microscope (Olympus) was used. Similar growth studies were done on the 5637 lines. Cell counts were taken on days 1, 2, 3, 4, 5, 8 and 9.

Results

Morphology: Cell outgrowth was seen within 24 h in the form of clusters mainly around the original tissue piece. It required about 35 days to see definitive growth and very few colonies were left by this time. By 65–80 days growth rate increased and cells were passaged every 4–6 days. The cells were epitheloid and irregular with large nuclei and multiple nucleoli.

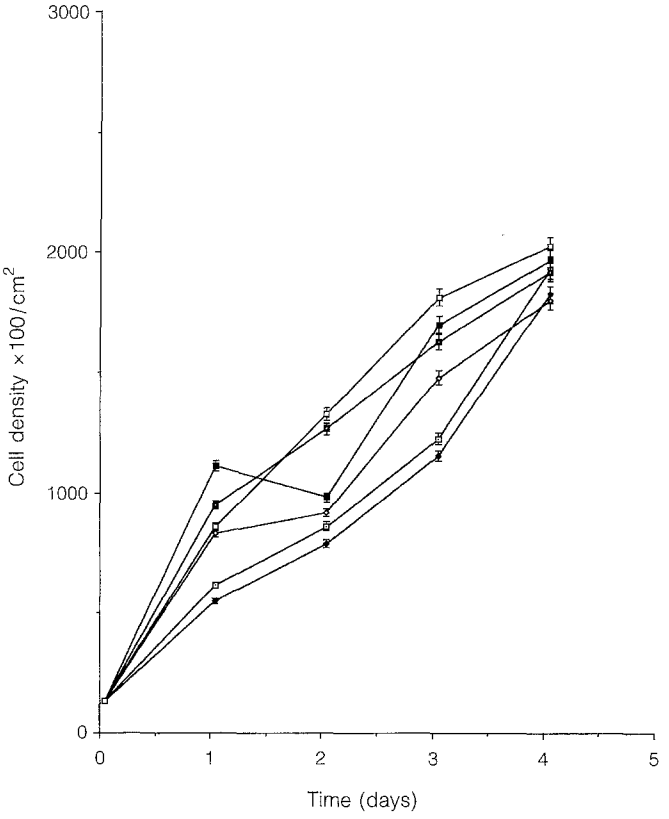

Fig. 1 Growth of 5637 cells.
—⊙— All; —□— basal + serum; —◆— basal; —◘— basal + selenium; —◇— basal + hydrocortisone; —■— basal + testosterone

Cytology: Cells showed large peripheral hyperchromatic nucleus with basophilic cytoplasm.

Electron microscopy: The cells had microvilli, ribosomes and desmosomes.

Cytokeratin staining: The DR cells showed patchy staining with cytokeratin antibody 18.

The growth pattern of the established cell line 5637 was found to be minimally affected by the medium supplements as shown in Fig. 1. There was no change in the morphology of cells. The cells became confluent after 72 hours.The growth of the early passage cell line DR (Fig. 2) was maintained only in serum containing medium. The growth was enhanced when other components were added. Basal medium on its own was not sufficient to maintain cell propagation. Only testosterone affected growth slightly but failed to maintain growth of the cells subsequently.

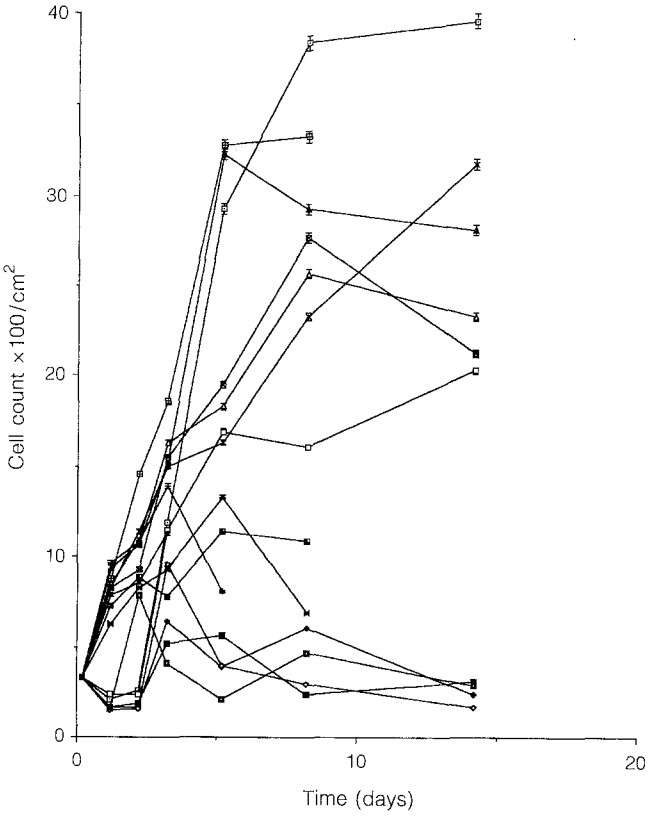

Fig. 2 Growth of DR cells.
—⊙— All; —△— B + S + Se; —□— B + Se; —◆— B; —▲— B + S + T; —⊠— B + H + S; —■— B + T + Se + H; —◇— B + H; —▬— B + T; —▩— B + Se + T + S; —✕— B + Se + T; —□— B + S; —✕— B + Se + H + S; —□— B + T + H

Discussion

Serum enhances the growth of early passage cell lines because it contains necessary nutrients and growth factors. Serum may be reduced or omitted from the medium if hormonal and nutritional requirements are optimized by the addition of specific components according to the needs of individual cell lines [12]. However, it is also known that serum may also contain substances which inhibit cell multiplication [11]. In the case of the cell line DR, serum enhanced the growth of the cells. Addition of serum did not make any significant difference to the growth of 5637 cells. In fact the 5637 cells will grow in virtually any medium. This is an important characteristic because established cell lines are used in the study of cellular proliferation and cell differentiation. Application of pharmacological responses from established cell lines is to a certain extent questionable because of these biological differences.

The growth requirements for established cell lines differ vastly from those of early passage cell lines. Even the basic features of epithelial cells like keratin expression may change when cells are transferred to long term in vitro conditions [1]. When cells are passaged continuously over a long period of time, one particular subpopulation of cells survive and show adaptability to the medium. The early passage cell lines on the other hand do not in general survive in unspecified medium because of their heterogeneous cell population. Therefore the biology of early passage cell lines may more closely resemble the cells of the tumour in vivo. This is supported by results of chemosensitivity/resistance assays on early passage cell lines which are known to reflect patterns of disease specific activity [9].

However, amplification of individual tumours by establishing early passage lines is a time-consuming and expensive exercise. The growth of cells beyond the initial period is difficult to achieve. It is most likely that early passage lines during this initial period represent the original tumour.

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