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Original Paper

Carcino-embryonic Antigen in Monitoring the Growth of Human Colon Adenocarcinoma Tumour Cells SK-CO-1 and HT-29 *In Vitro* and in Nude Mice

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A set of experimental model systems were designed to investigate (a) the inter-relationship between growth of two human cancer cell lines (SK-CO-1, HT-29) and carcino-embryonic antigen (CEA) kinetics; and (b) whether neoplastic growth or CEA concentration is modulated by human growth hormone (hGH). We found that increasing CEA concentration depended on tumour burden. SK-CO-1 cells had the lowest growth rates but the highest rates of CEA production. The rate of CEA increase exceeded the growth rate of both SK-CO-1 and HT-29. hGH modulated neither neoplastic growth nor CEA production. In conclusion, our results suggest that experimental models may be useful for investigating the role of serological markers as monitors of increasing tumour burden. It will be of interest to investigate the performance of those model systems in examining the effect of cytotoxic agents in neoplastic growth.

Key words: carcino-embryonic antigen, tumour cells, cultured, *in vitro*, mice, nude

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INTRODUCTION

IN THE last 20 years, numerous clinical studies have investigated whether serological tumour markers reflect tumour burden [1]. However, the interpretation of marker signals is hampered by variations in concentrations discordant with the clinical activity of disease [2].

Laboratory experiments might prove valuable for investigating the effect of neoplastic growth on tumour markers since, in these systems, tumour burden can be determined with higher accuracy than in patients [3–5]. If marker concentration depends on tumour burden, then the markers may be of use in investigating the effects of different antineoplastic agents because the agents can be assessed not only in terms of cell growth, but also biochemically in terms of marker production. Recent studies have suggested that administration of human growth hormone (hGH) after surgery for cancer could have valuable practical applications by improving postoperative convalescence and wound healing [6]. However, controversy exists over its possible stimulatory effect on tumour growth [7].

The possibility of involving tumour markers in experimental studies have improved as an increasing number of marker-producing human neoplastic cell lines, mostly carcino-embryonic antigen (CEA) producers, have been identified [8–12]. CEA belongs to a group of heterogeneous glycoproteins which are members of the immunoglobulin supergene family expressed in a variety of malignant and non-malignant tissues, and may participate in intercellular recognition and attachment processes [13].

The purpose of the present study was to investigate the performance of a set of model systems for assessing: (a) the

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inter-relationship between growth rate of human colon cancer cells and rate of CEA release into the culture medium; (b) the inter-relationship between tumour growth rate and rate of CEA release into the serum of nude mice transplanted with human colon cancer cells; and (c) whether growth of neoplastic cells or rate of CEA release was modulated by hGH.

MATERIALS AND METHODS

Cells

Human colon adenocarcinoma cell lines SK-CO-1 and HT-29 (from the human tumour cell bank, Sloan-Kettering Institute for Cancer Research, U.S.A.) were used. SK-CO-1 has been isolated from ascites of a patient with colon carcinoma, and HT-29 has been isolated from a primary tumour in the colon [14]. The cells were cultured (T175 flasks, Nunc, Denmark) in MEM with 10% fetal calf serum (FCS), penicillin (100 kU/l), streptomycin (100 mg/l), L-glutamine (2 mM) and sodium bicarbonate (2 g/l) (C-MEM) in 5% CO_2 and 95% air at 37°C. The cells were used in all experiments at passage numbers between +1 to +20. Medium, sera and antibiotics were obtained from Gibco, U.S.A.

Mice

BALB/C nude mice (BOMMICE, Denmark) were kept in sterile conditions on Hahnflock pine bedding and fed Altromin 1324. The cage, filtertop, bedding and feed were all heat treated at 60°C for 4 h prior to use. The room temperature was 21 +/− 1°C and the humidity was 60 +/− 10%. Blood specimens were obtained by orbital venipuncture and serum was separated by centrifugation at 1000 *g* for 20 min.

Study design

In preliminary *in vitro* experiments, we investigated whether SK-CO-1 and HT-29 cells produced CEA. As HT-29 did not produce CEA, further *in vitro* experiments focused on SK-CO-1. Preliminary experiments showed that both cell lines produced CEA when transplanted into nude mice. Consequently, further *in vivo* experiments included both SK-CO-1 and HT-29 cells.

The inter-relationship between exponential growth of SK-CO-1 cells and CEA concentration in the culture medium was determined in four series of 12 wells. Cells from 80% confluent flasks were trypsinised and plated (Nunc, Denmark) with a density of 2×10^4 cells per ml in C-MEM with 10% FCS and incubated in 5% CO_2 and 95% air at 37°C. The cells were cultured without a change of medium. Every 24 h, the growth in one series of wells was stopped and the CEA concentration in the medium determined individually for each well following centrifugation. The cells were trypsinised and the number of viable cells counted in a haemocytometer using trypan blue.

In vivo, the inter-relationship between tumour growth and serum CEA concentrations was determined in 30 5–6 week-old mice inoculated subcutaneously in the neck with a pellet containing 6×10^6 SK-CO-1 cells, and in 30 mice inoculated with 6×10^6 HT-29 cells. Fifteen animals in each group received hGH (Norditropin, Novo Nordisk, Denmark); the rest placebo. Groups dosed with hGH were injected daily with 1 IU/kg in a buffer containing 1% albumin. Groups receiving placebo were injected with the buffer only.

Growth analysis

In vitro, doubling time (DT) of cells was calculated as: $((t_{(2)} - t_{(1)}) \times \ln 2)/(\ln Q_{t(2)} - \ln Q_{t(1)})$ [15]. However, we also applied the equation for calculating the DT of CEA. $Q_{t(1)}$ was the cell number or the CEA concentration at time $t_{(1)}$, and $Q_{t(2)}$ was the cell number or the CEA concentration at time $t_{(2)}$. In mice, Gompertz tumour size and tumour volume were calculated from two perpendicular tumour diameters as described previously [16]. Doubling time of tumours was computed on basis of a linear transformation of the Gompertz growth function [16], whereas DT of CEA in mice was calculated with the same equation as was the DT of CEA in the culture medium.

Calculation of the CEA production per SK-CO-1 cell per 24 h in vitro

Previous studies have estimated marker production by dividing the marker concentration in the culture medium with the cell number [11, 17]. This approach merely expressed the marker concentration per cell at a given time point. To calculate the genuine marker production per cell per time unit we derived a set of equations provided in the Appendix section.

Determination of CEA

CEA was determined using the CEA DELFIA (Wallac, Finland), a commercially available solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique. The minimal detectable concentration (MDC) 0.01 µg/l was calculated with the MultiCalc computer package (Wallac, Finland). *In vitro* samples were assayed in duplicate, whereas serum CEA assays were performed as single determinations. Serum samples with volumes less than 25 µl were diluted prior to analysis. Concentrations above the MDC were multiplied with the dilution factor.

Statistical analysis

Data analysis was performed with the Statgraphics computer package, version 2.6 (STSC, U.S.A.). We used linear regression analyses for assessing neoplastic growth as a function of time, CEA increase as a function of time, and CEA increase as a function of neoplastic growth. The package provided the rate of change (slope) of the regression line, the coefficient of correlation, and the *P*-value for the correlation.

In further statistical analyses, we investigated (a) whether CEA concentrations in the culture medium increased faster than the cell number by comparing the rates of CEA increase with the growth rates of SK-CO-1 cells; (b) whether hGH influenced tumour growth by comparing the Gompertz growth rates of hGH and placebo treated mice; (c) whether hGH influenced CEA production by comparing the rates of CEA increase of hGH and placebo treated animals; (d) whether SK-CO-1 or HT-29 tumours grew fastest by comparing the Gompertz growth rates of placebo treated hosts; (e) whether SK-CO-1 or HT-29 tumours had the highest CEA production by comparing the rates of CEA increase of placebo treated mice; and (f) whether CEA concentrations increased faster than the tumour volumes by

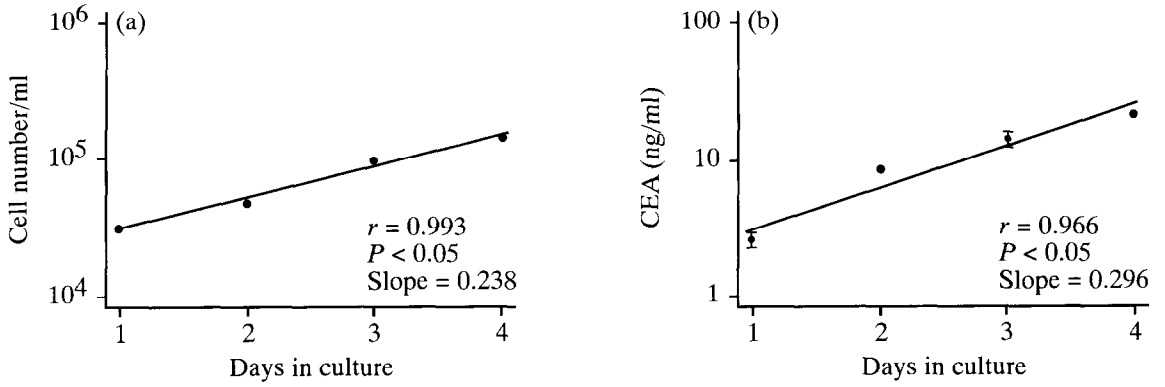

Figure 1. SK-CO-1 cell number (a) and CEA concentration (b) in the culture medium as a function of time. Points, mean; bars, S.E.M.; S.E.M. is not shown when it does not exceed the symbol size.

comparing the rates of CEA increase with the tumour volume growth rates in placebo treated animals.

All comparisons of slopes were performed with the Mann–Whitney test. The mean CEA production *in vitro* per SK-CO-1 cell per 24 h was determined on 4 successive days and compared with the Kruskal–Wallis test. A P value <0.05 was considered to indicate statistical significance.

RESULTS

In vitro *experiments*

During an observation period of 4 days, the number of SK-CO-1 cells and the CEA concentrations increased exponentially without any observed dead cells (Figure 1a, 1b). The rates of CEA increase exceeded the growth rates of SK-CO-1 cells ($P < 0.05$) suggesting that CEA concentrations increased faster than the cell number. Correspondingly, the DT was 1.0 day for CEA and 1.3 days for SK-CO-1, the DT of CEA being 30% faster than of cells. The linear relation between SK-CO-1 cell number and CEA concentration is provided in Figure 2, and shows that the CEA concentration depended upon the number of SK-CO-1 cells.

During exponential growth marker production per cell per time unit should be similar at different time points in a homogeneous sample of cells. The mean CEA production per SK-CO-1 cell per 24 h was approximately constant throughout the monitoring period, 1.13×10^{-4} ng on day 1, 1.34×10^{-4} ng on day 2, 1.01×10^{-4} ng on day 3, and 0.92×10^{-4} ng on day 4 ($P > 0.05$). The overall mean CEA production per SK-CO-1 cell per 24 h calculated for all 4 days was 1.11×10^{-4} ng $\pm$ 0.07×10^{-4} ng (S.E.M.).

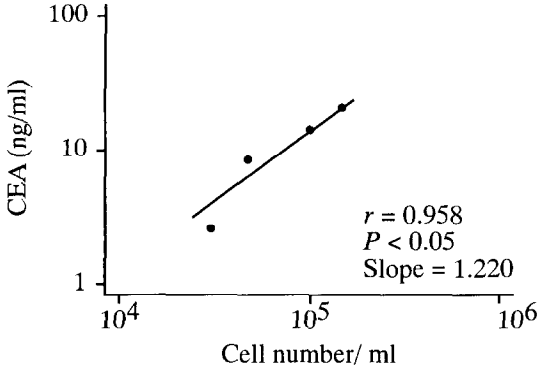

Figure 2. Mean CEA concentration in the culture medium as a function of mean SK-CO-1 cell number.

In vivo *experiments*

Ten mice were excluded because of a lack of tumour growth or early death. In the remaining 50 animals, the tumour growth fitted well to a linearly transformed Gompertz function (Figure 3a–d). For a comparison the tumour volume as a function of time is provided in Figure 4a–d). Tumour growth was not influenced by hGH since the Gompertz slopes in the placebo- and hGH-treated hosts were identical ($P > 0.05$). The Gompertz slopes of placebo-treated HT-29 tumours exceeded those of placebo-treated SK-CO-1 tumours ($P < 0.05$) suggesting that HT-29 tumours grew faster than SK-CO-1 tumours. Correspondingly, the DT was 4.9 days for HT-29 and 6.7 days for SK-CO-1, the DT being 37% faster for HT-29 tumours. The slower growth rate partly explained the longer lag-period of SK-CO-1.

The CEA concentrations increased exponentially (Figure 5a–d). Growth hormone had no influence on production, since the rates of CEA increase in the placebo- and hGH-treated mice were almost identical ($P > 0.05$). The rates of CEA increase of placebo treated SK-CO-1 tumours exceeded those of placebo-treated HT-29 tumours ($P < 0.05$) suggesting that SK-CO-1 tumours had a higher CEA production rate than HT-29. Correspondingly, the CEA DT was 2.1 days for SK-CO-1 tumours and 2.4 days for HT-29, the SK-CO-1 tumours producing CEA 14% faster than HT-29.

The serum CEA concentration depended upon tumour volume (Figure 6a–d), but the rates of CEA increase exceeded the tumour volume growth rates ($P < 0.05$) suggesting that CEA concentrations increased faster than tumour volumes. Based upon the placebo treated animals, the DT of CEA was more than 3-fold faster than the DT of SK-CO-1 tumours and twice as fast as the DT of HT-29 tumours.

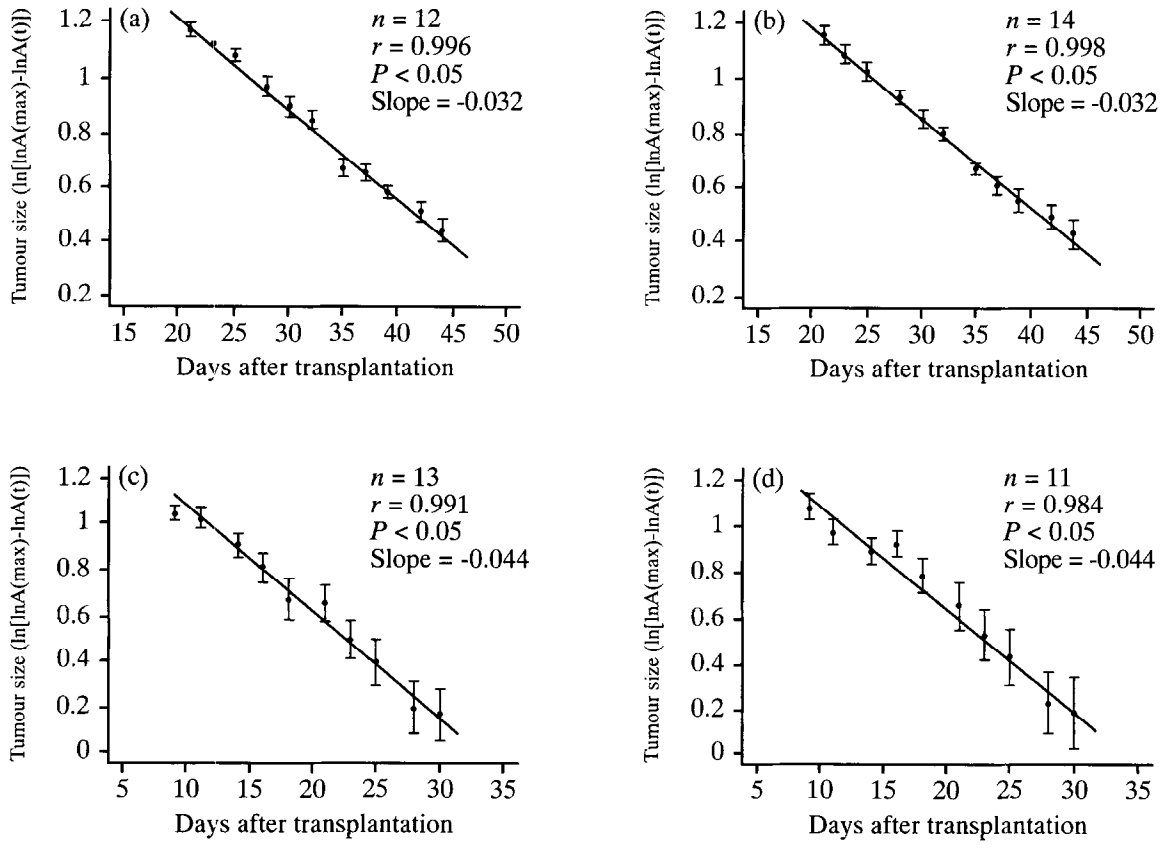

Figure 3. Transformed Gompertz tumour size as a function of time for two colon cancer xenografts, SK-CO-1 and HT-29, treated with placebo or growth hormone: (a) SK-CO-1 placebo; (b) SK-CO-1 plus growth hormone; (c) HT-29 placebo; (d) HT-29 plus growth hormone. Points, mean; bars, S.E.M.; S.E.M. is not shown when it does not exceed the symbol size. *n*, number of mice in each group.

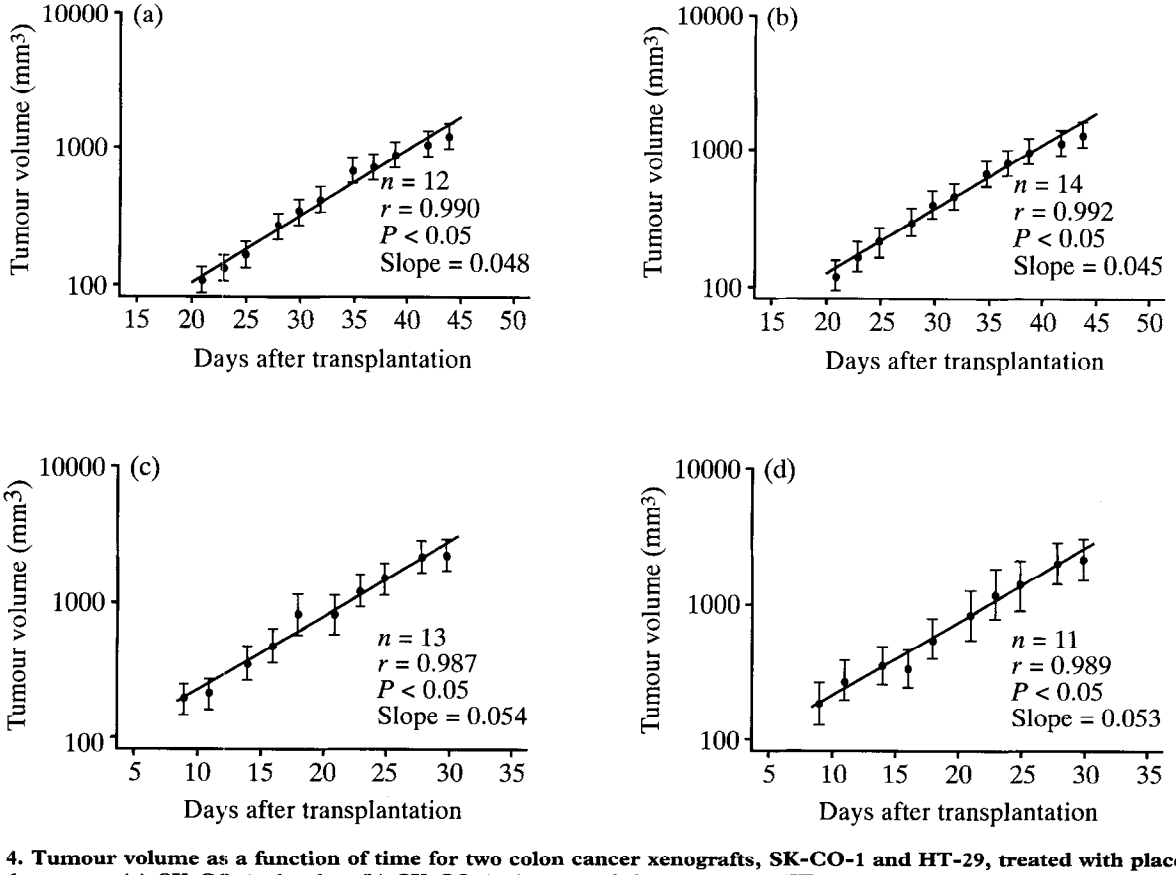

Figure 4. Tumour volume as a function of time for two colon cancer xenografts, SK-CO-1 and HT-29, treated with placebo or growth hormone: (a) SK-CO-1 placebo; (b) SK-CO-1 plus growth hormone; (c) HT-29 placebo; (d) HT-29 plus growth hormone. Points, mean; bars, S.E.M. *n*, number of mice in each group.

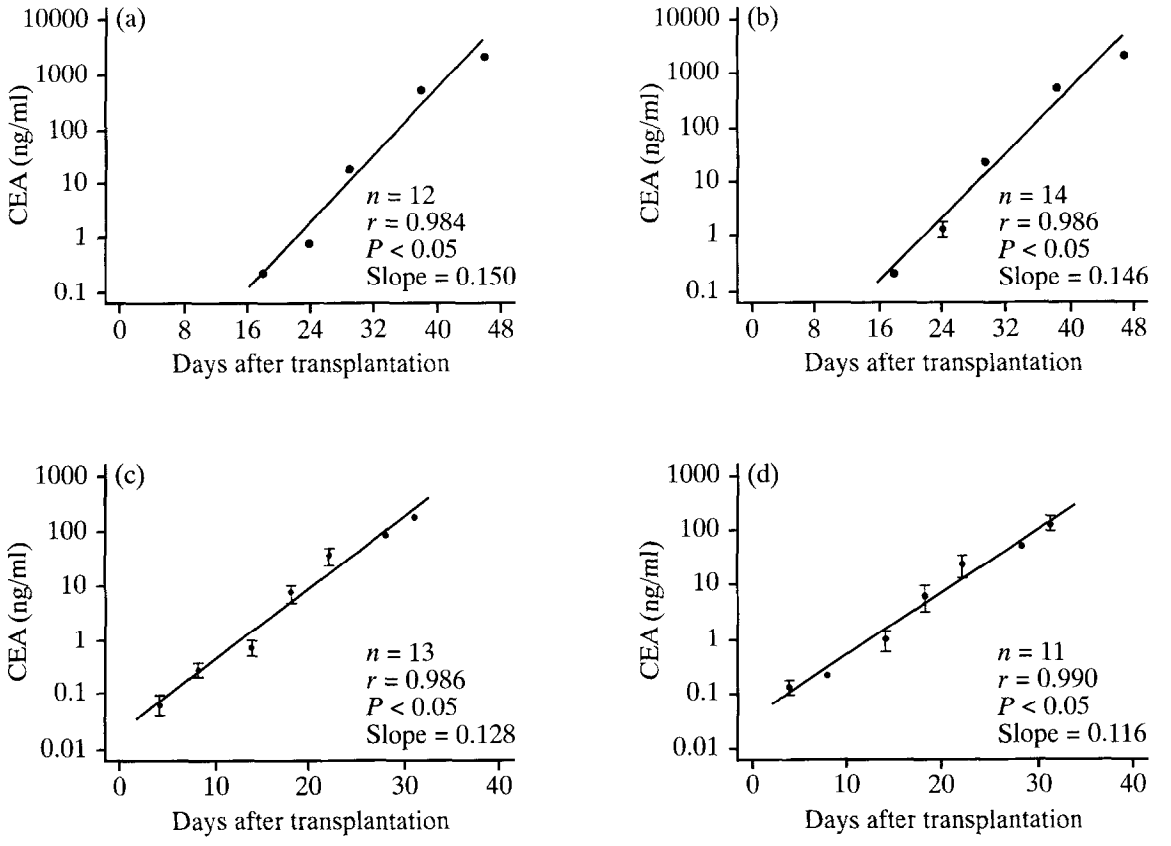

Figure 5. Serum CEA concentration as a function of time for mice transplanted with human colon cancer cells, SK-CO-1 or HT-29, and treated with placebo or growth hormone: (a) SK-CO-1 placebo; (b) SK-CO-1 plus growth hormone; (c) HT-29 placebo; (d) HT-29 plus growth hormone. Points, mean; bars, S.E.M.; S.E.M. is not shown when it does not exceed the symbol size. *n*, number of mice in each group.

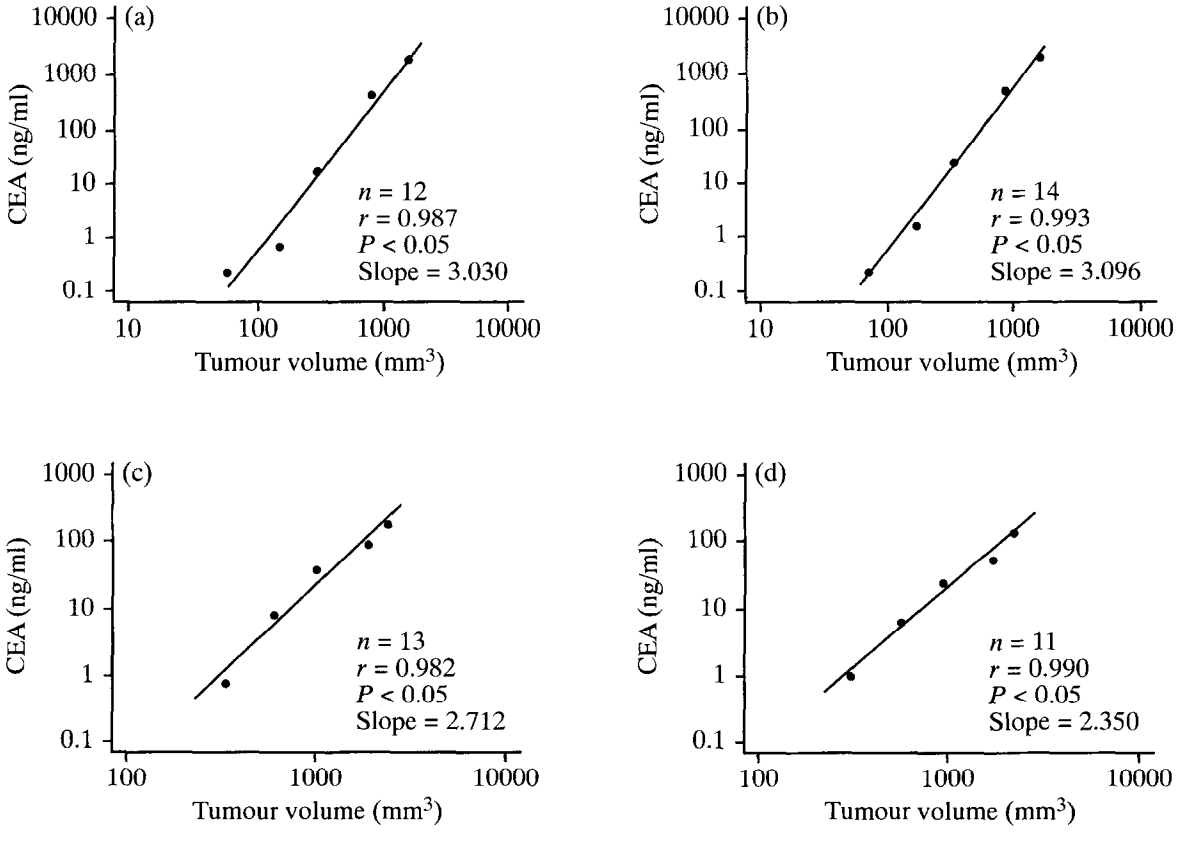

Figure 6. Mean serum CEA concentration as a function of mean tumour volume for mice transplanted with human colon cancer cells, SK-CO-1 or HT-29, and treated with placebo or growth hormone: (a) SK-CO-1 placebo; (b) SK-CO-1 plus growth hormone; (c) HT-29 placebo; (d) HT-29 plus growth hormone. *n*, number of mice in each group.

DISCUSSION

In this paper, we have described the performance of a set of experimental model systems for monitoring two human tumour cell lines, SK-CO-1 and HT-29. The results support our assumption that CEA could be used for monitoring increasing tumour growth in experimental systems because increasing CEA concentrations depend upon increasing tumour burden. Our model systems may prove useful for a more detailed study of tumour markers as compared to previous investigations because the systems allow not only a comparison of tumour growth rates, but also a comparison of rates of marker increase, as well as a comparison of tumour volume growth rates with rates of marker increase. We found that SK-CO-1 tumour had the lowest growth rate but the highest rate of CEA production, and that the rate of CEA increase exceeded the growth rate of both SK-CO-1 and HT-29. Growth hormone modulated neither cell proliferation nor CEA secretion.

Studies on the inter-relationship between cell number and marker concentration are scarce and the degree of correlation has remained unclear. Previous reports have had either a short observation period (24 h) or related the marker concentration in the culture medium to time and not to cell number [10, 17]. We found that the CEA concentration in the culture medium depended upon the SK-CO-1 cell number during exponential growth (Figure 2). It could be speculated that release of intracellular CEA from dead and disintegrating cells contributed significantly to the concentrations measured in the medium. However, this was not likely because SK-CO-1 cells remained viable throughout the monitoring period and because the mean CEA production per cell per time unit, as calculated from the cell numbers and CEA concentrations, remained approximately constant. The relationship between cell growth and marker concentration will probably be difficult to investigate in the post-exponential growth phase because the CEA concentration will depend on production in viable cells as well as on release from the large fraction of dead and disintegrating cells characteristic of post-exponential growth. It is relevant to ask why HT-29 cells released CEA *in vivo* but not *in vitro*. A reasonable explanation is that CEA production in HT-29 cells may depend on the composition of the culture medium. Our observation on this issue is in agreement with a previous report [17].

The close inter-relationship *in vivo* between marker increase and tumour growth in our study is strengthened by the fact that these observations are based on a reasonable number of mice (Figure 6a–d). Two previous studies on the utility of serological markers as tumour monitors were based on only a few mice. One study reported a relationship between subcutaneous growth of human colorectal cancers and serum CEA [18]; the other study reported a relationship between subcutaneous growth of human prostate LNCapP tumours and serum prostate-specific antigen levels [9]. Taken together, the three studies suggest that nude mice models may be useful for investigating the role of serological markers as monitors of increasing tumour burden. However, a standardised procedure for transplantation is probably a precondition because discordance between tumour growth and marker concentration has been reported frequently in experiments applying tumour fragments or multiple implants [19, 20].

We have focused on the ability of CEA in monitoring increasing tumour burden. One of the next steps in our investigation will be to analyse whether CEA reflects tumour regression when development is impaired by an agent significantly modifying neoplastic growth. However, determination of circulating CEA shortly after administration of a cytotoxic agent should be avoided because of transient increments associated with tumour lysis [21]. If CEA also reflects decreasing tumour burden, marker monitoring may be valuable as a supplement to preclinical studies because assessment of an agent; efficacy can be uncertain due to undetectable tumours and a few neoplastic cells in fibrotic or necrotic residual sites.

Our observations indicate that experimental marker kinetics may explain marker kinetics in patients. Thus, the variability in serological marker concentrations among patients with similar tumour burden, and the varying ability of markers to provide early information about progressive disease, may partly be explained by different rates of marker production by different cell clones (Figure 5a–d).

In conclusion, studies investigating what can be achieved in the presented model systems with agents significantly modulating neoplastic growth will be of considerable interest. Additionally, it will be of interest to investigate whether the systems can be used for preclinical screening of different cancer cell lines to determine whether they can produce a new potential marker, and in determining the ability of a potential marker to reflect tumour burden.

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APPENDIX

Calculation of the CEA production per SK-CO-1 cell per 24 h in vitro

Assuming that the number of cells were $N_{(0)}$ at time $t_{(0)}$, and that the growth rate was (b), the cell number at time (t) was

$$N_{(t)} = N_{(0)} \times e^{b \times t} \text{ [15]} \quad (1)$$

If one cell produced (m) ng CEA per time unit, the amount of CEA (C) increased from time (t) to time ($t + dt$) with:

$$dC_{(t)} = N_{(t)} \times m \times dt = N_{(0)} \times e^{b \times t} \times m \times dt \quad (1b)$$

The amount of CEA produced from time $t_{(0)}$ to time (t) was

$$\begin{aligned} C_{(t)} &= \int_0^t dC_{(t)} \times dt = \int_0^t N_{(0)} \times e^{b \times t} \times m \times dt \\ &= N_{(0)} \times m \times \left[1/b \times e^{b \times t}\right]_0^t = N_{(0)} \times m/b \times (e^{b \times t} - 1). \end{aligned} \quad (2)$$

$C_{(0)}$ was zero as CEA production started at $t > t_{(0)}$. Equation (2) was inserted into equation (1):

$$N_{(t)} = C_{(t)} \times b/m \times (e^{b \times t}/(e^{b \times t} - 1)) \quad (\text{for } t > 0). \quad (2b)$$

Consequently, the CEA production per cell per time unit (m) is

$$C_{(t)}/N_{(t)} \times b \times e^{b \times t}/(e^{b \times t} - 1). \quad (2c)$$