Eur J Cancer Clin Oncol, Vol. 21, No. 7, pp. 833-843, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Characterization of the T61 Human Breast Carcinoma Established in Nude Mice*

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Abstract—*This paper gives a biological characterization of the T61 human breast carcinoma established in nude mice. The human origin of the tumour was verified by the demonstration of the presence of human chromosomes exclusively in the tumour cells. The tumour cells were found by chromosome analysis and flow cytometric DNA analysis to be aneuploid. By electron microscopy, the tumour cells were shown to display the characteristics of glandular epithelium; a light microscopic examination revealed morphological characteristics similar to those of an axillary metastasis of the patient from whom the T61 tumour was derived. Furthermore, the tumour was shown to contain classical receptors for oestrogen and progesterone. The growth of the tumour was characterized by gompertzian growth curves. Since the T61 tumour has a response pattern to endocrine treatment which differs from that described for other human breast tumours grown in nude mice, this tumour may provide a valuable supplement in the study of human breast cancer and endocrine treatment.*

INTRODUCTION

THE ABILITY of nude mice to accept human tumours and the preservation of many human tumour characteristics even after many passages in nude mice [1-3] make this model system well-suited for the study of human tumours under *in vivo* conditions.

A number of human breast carcinomas have been established in nude mice [4-8]. One of these, the T61 tumour, has a response pattern to endocrine treatment [8, 9] which differs from that described for other human breast carcinomas grown in nude mice [5, 6] but is similar to the endocrine response patterns of the R3032AC rat mammary tumour [10] and to responses observed in some clinical breast tumours [11,12]. These data suggest that the T61 tumour may provide a valuable supplement in the study of the effect of endocrine treatment on the growth of human breast cancer.

The present paper describes the biological characteristics of the T61 human breast tumour grown in nude mice. The tumour was characterized by chromosome analysis, flow cytometric DNA analysis, light and electron microscopy, oestrogen and progesterone receptor assay, and gompertzian growth curves.

MATERIALS AND METHODS

Tumour

The T61 tumour was obtained from the surgical specimen of the primary breast cancer of a 54-year-old post-menopausal woman who underwent a mastectomy in 1974 for an invasive ductal carcinoma (WHO grade II) of the right breast (T_1,N_-,M_0). The patient died from progressive disease in 1977. By autopsy, the tumour was found to have disseminated to both axillae, the mediastinum, pleura and hepar. The only data

Accepted 25 October 1984.
*This study was supported by grants from the Danish Cancer Society, the Thaysen Foundation, and the Foundation of 1870.
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available on the patient tumour are the morphology of the primary tumour and the morphology of a lymph node metastasis removed from the ipsilateral axilla 3 yr after the primary operation.

A biopsy of the primary breast cancer specimen was transplanted without any culture procedures into castrated nude mice. This specimen gave rise to the continuous serial transplantable tumour described in this report.

The tumour was established in Frankfurt am Main, F.R.G. Then, in the 11th passage in nude mice, the tumour was transported to Copenhagen. The present data are derived from tumours at passages 1-27 in nude mice.

For serial growth, a tissue block of about 2 mm was transplanted subcutaneously into each flank of the animals, aged 6-8 weeks. Surgical procedures were performed under general anaesthesia with propanidid (Epontol®).

Chromosome analysis

Tumour tissue from two tumours grown in castrated male nude mice, passage 21, was minced with scalpels and incubated for 2 hr at 37°C in Hams F_{10} supplemented with 20% foetal calf serum (FCS) and 20 μg/ml collagenase (Sigma, type I) [13]. After several rinses in PBS, the dispersed cells were incubated at 37°C in F_{10} and 20% FCS for 48 hr. Two hours prior to harvest, colcemid (0.04 μg/ml) and ethidium bromide (5 μg/ml) were added. Hypotonic treatment (KCl), fixation (methanol:acetic acid = 3:1) and the preparation of air-dried slides followed routine procedures. Chromosomes were Q-banded with quinacrine mustard [14] and G-banded with Wright's stain [15]. Photos were taken of a total number of 18 well-spread metaphases for analysis.

Histology

Tumours from passages 1-27 in nude mice were examined histologically. Sections were stained with haematoxylin and eosin, or by the van Gieson method. The histology of the mouse-grown tumours was compared with histological sections of the primary patient tumour and with sections of the lymph node metastasis removed 3 yr after the mastectomy.

Electron microscopy

Biopsies from tumours grown in castrated male nude mice, passages 21-23, were fixed for 2 hr in 70% Karnovsky's solution, and then processed for electron microscopy according to the routine procedures at the laboratory [16]. Appropriate areas for electron microscopy were selected by a light microscopy examination of 1 μm sections stained with toluidine blue. Ultrathin sections were cut with an LKB Ultrotome equipped with a diamond knife. The sections were stained with uranyl acetate and lead citrate with an LKB Ultrostainer and examined with a Philips 201 electron microscope.

Receptor assay

Oestrogen and progesterone receptor assays were performed on samples obtained from tumours growing on mice of both sexes. Fifty tumours from passages 11-27 were analysed. The samples were frozen immediately after excision and stored at -80°C until analysed. The receptor analysis was performed according to the procedures described elsewhere [17-19]. Briefly, the dextran-coated charcoal assay (DCC) was applied in order to characterize the binding capacity and affinity of oestrogen and progesterone receptors. In addition, a sucrose density gradient ultracentrifugation (SDGU) was performed to characterize the macromolecular forms of the high-affinity steroid bindings.

Flow cytometric DNA analysis

The analysis was performed on tumour tissue obtained by fine-needle aspiration. Biopsies from several hundred tumours in passages 11-27 were analysed. The aspiration procedure, the storage of aspirates, and staining with propidium-iodide were performed as described elsewhere [20-22]. The flow cytometer used was a FACS III cell sorter (Becton Dickinson, Sunnyvale, CA). The cellular DNA content was expressed by the DNA index (DI), defined as the ratio of the DNA content of the tumour G_1 cells to that of diploid human cells. For the determination of the DI, chicken red blood cells and trout red blood cells were used as internal standards [22]. The percentage of cells in the cell cycle phases was determined by the statistical analysis of the DNA distributions [23].

Growth curves

The macroscopic growth of the tumour was studied in male and female nude mice. Several hundred tumours in passages 12-27 were examined. The 'length' and the 'width' of the tumours were measured three times a week, and the 'height' once a week [24]. The tumour area was calculated as the product of the 'length' and 'width' of the tumour. The tumour area was then used to construct rectilinear growth curves according to a transformed Gompertz function [24]:

$$\ln(\ln A_{max} - \ln A(t)) = \ln \beta/\alpha - \alpha t,$$

where $A(t)$ is the tumour area at time t, α and β are constants and A_{max} is the mean theoretical

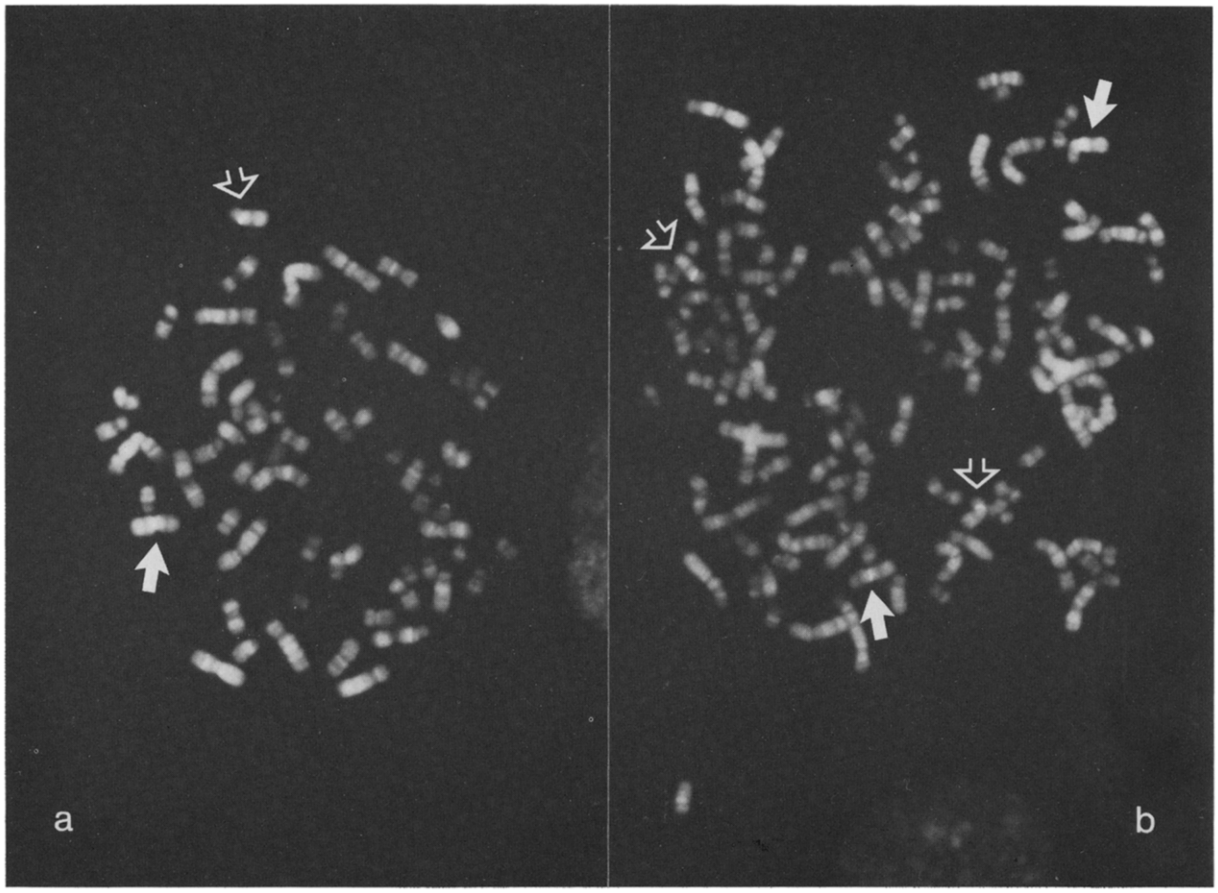

Fig. 1. Q-banded metaphases from the T61 human breast carcinoma grown in nude mice, passage 21. (a) Hyperdiploid cell with 61 chromosomes. (b) Hypertetraploid cell with more than 120 chromosomes. Closed arrows indicate the normal chromosome 3; open arrows indicate the deleted chromosome 3 (3p-).

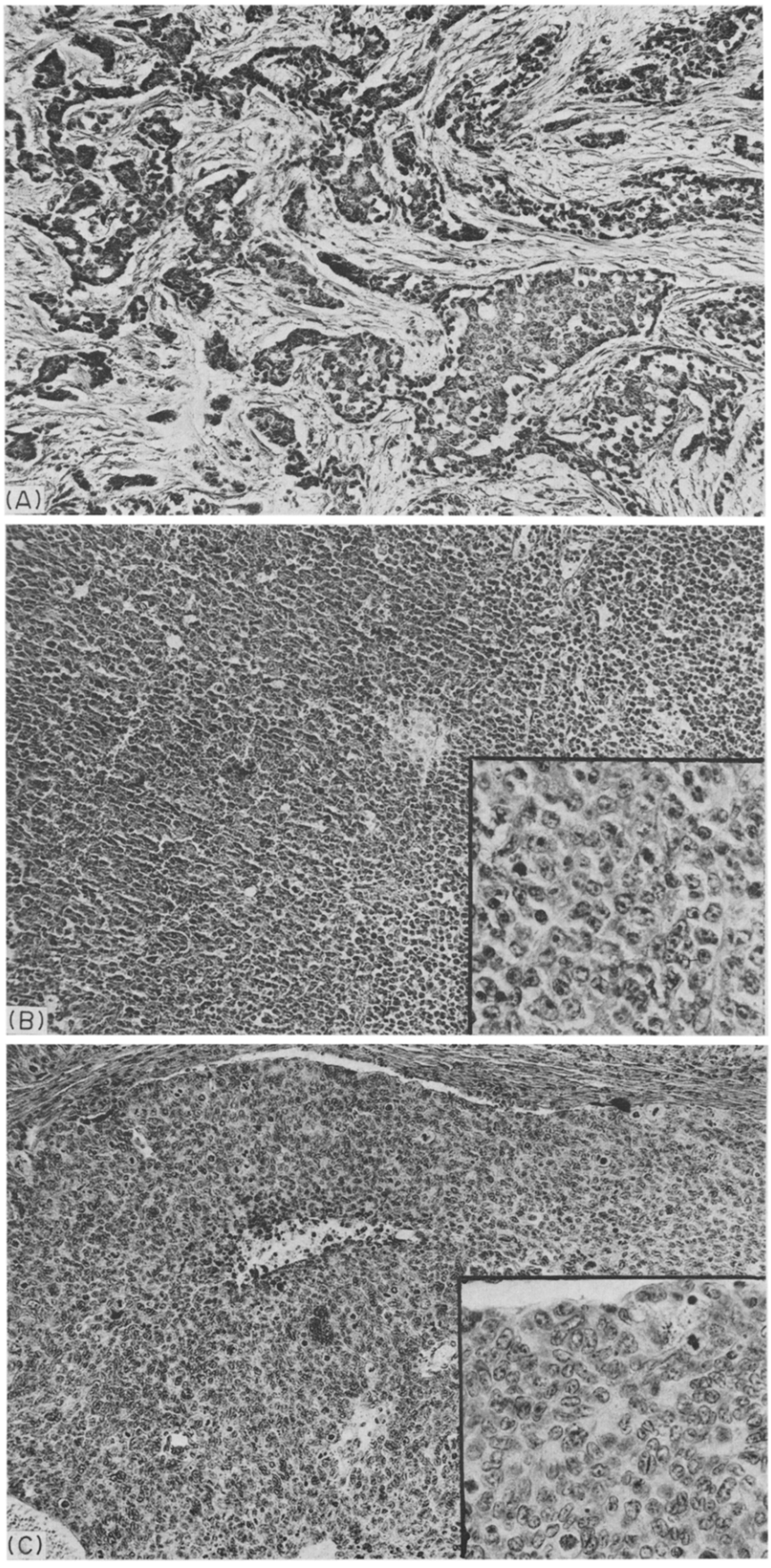

Fig. 2. Histological sections from the T61 human breast carcinoma. (A) Primary patient breast carcinoma. Moderately differentiated ductal carcinoma, WHO grade II. H-E × 184. (B) Axillary lymph node metastasis. The tumour tissue displays the characteristics of a medullary carcinoma. H-E × 184 and × 460. (C) The first passage in nude mice. The tumour showed a histological structure very similar to that of the metastasis shown in Fig. 2B. H-E × 184 and × 460.

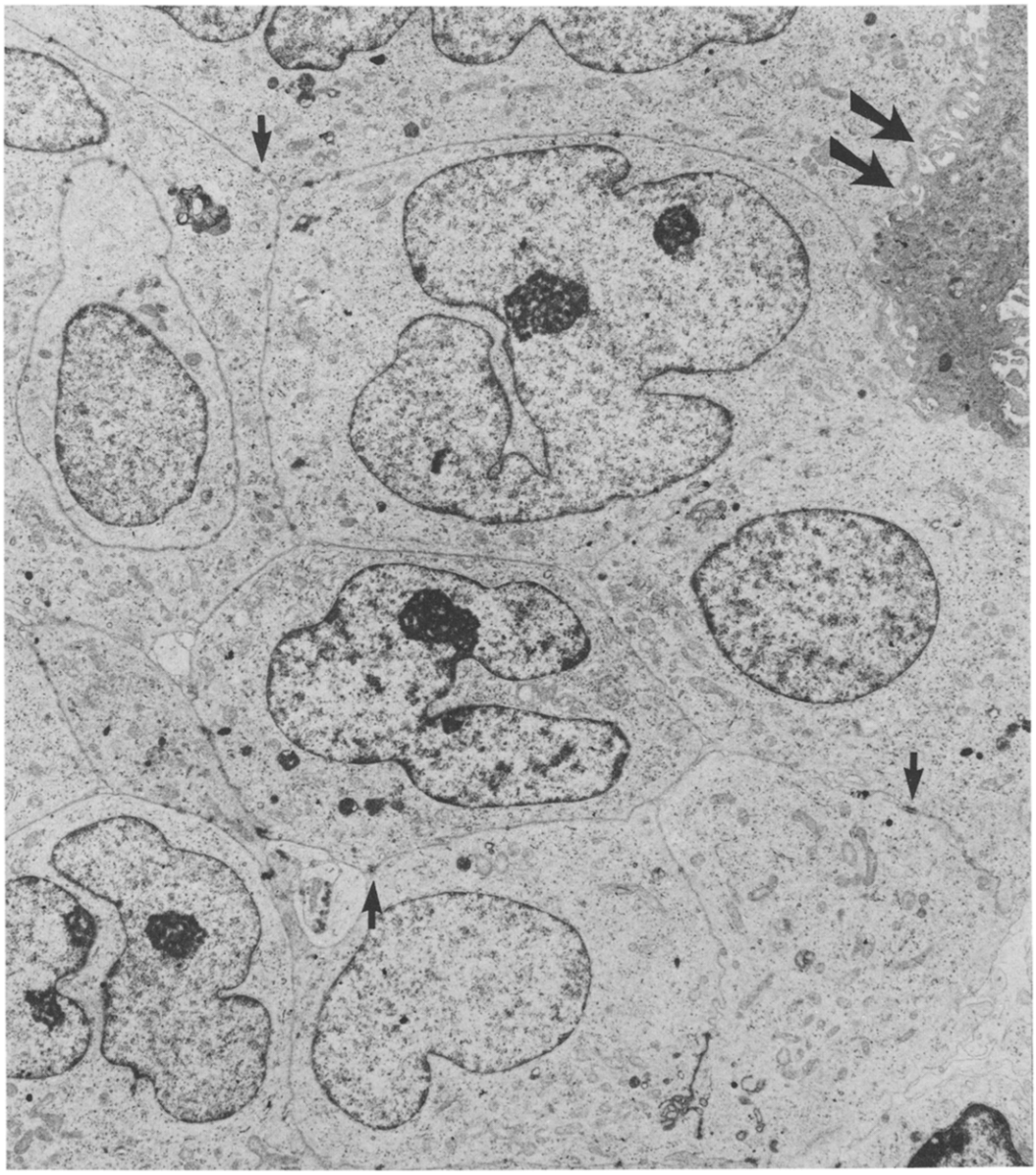

Fig. 3. Electron micrograph of the T61 human breast carcinoma grown in nude mice, passage 23. The nuclei show deep indentations and dense nucleoli. Note the numerous desmosomes (arrows) and the microvilli on the surface (double arrows). Uranyl acetate and lead citrate, original magnification × 7500.

maximum area. The value of $\ln A_{max}$ was graphically [24] estimated to be seven for this tumour. The transformed Gompertz function depicts the growth rectilinearly with the slope $-\alpha$ when the tumour size $\ln (\ln A_{max} - \ln A(t))$ is plotted as a function of time after transplantation.

RESULTS

The chromosome analysis revealed a heavily rearranged human karyotype (Fig. 1). Very few normal chromosomes could be identified. Thus, it was impossible to make a detailed characterization of the chromosome constitution.

Two populations were found (Fig. 1). One population (14 out of the 18 cells analysed) was hyperdiploid with modal number (mn) 61 (range 57–66). The other population (four out of the 18 cells) was hypertetraploid with mn 122 (range 117–126). By Q-banding, one normal chromosome 3 and one deleted chromosome 3 (3p–) were observed in the hyperdiploid population. In the hypertetraploid population, two of each of normal chromosome 3 and 3p– were seen (Fig. 1).

Figure 2 shows the histology of the tumour tissue from the patient (Fig. 2A and B) and after transplantation into nude mice (Fig. 2C). The tumour cells in the mouse-grown tumours (Fig. 2C) proliferated in solid sheets and had often very large, vesicular, rounded or elongated nuclei and small amounts of slightly basophilic cytoplasm. The tumours were surrounded by a thin connective capsule and only occasionally did they invade the related skeletal muscle. The morphology of the mouse-grown tumours did not change in the subsequent 27 passages.

The histology of the tumours in nude mice appears to be similar to that of the lymph node metastasis (Fig. 2B and C), but different from that of the primary tumour (Fig. 2A) from which it was originally transplanted (see Discussion).

Electron microscopic investigations showed that the tumour consisted of rather solid sheets of large pleomorphic cells (Fig. 3). The nuclei frequently displayed deep indentations and the chromatin appeared almost exclusively as euchromatin. The nuclei contained one or two dense nucleoli. The abundant cytoplasm of the neoplastic cells to varying degrees contained rough endoplasmatic reticulum, and free polysomes were numerous. In addition, intracytoplasmic vacuoles were observed in some cells. Well-developed desmosomes were always found with intermediate filaments (Fig. 3, arrows), but the latter were rare in the cytoplasm. Lateral foldings were often found when cells were apposing, and microvilli were prominent at the free surface (Fig. 3, double arrows).

Figures 4 and 5 show that saturable, high-affinity and ligand-specific binding of oestrogen and synthetic progesterone could be demonstrated in cytosols obtained from the T61 tumour. Figure 4 shows that the high-affinity binding of oestrogen was confined to a small 4S and a larger 8S peak in the SDGU assay, and Fig. 5 reveals that the high-affinity binding of synthetic progesterone was primarily confined to the 8S area. Thus, although some variation was found in the receptor content of individual tumours, the results unequivocally demonstrated classical oestrogen and progesterone receptors in the T61 tumour [25].

Figure 6 gives the results of a representative flow cytometric DNA analysis of the T61 tumour. The histogram demonstrates one aneuploid tumour cell population with a DI of 1.4. The calculated cell cycle distribution ± S.D. of 18 tumours in passage 23 was G_1: 60.48 ± 4.02, S: 36.11 ± 3.72 and G_2 + M: 3.39 ± 1.41. In addition to this tumour cell population, a small fraction of polyploid cells without distinct peaks appears in the histogram to the right of the G_2+M peak (Fig. 6). Although some variation was found in the cell cycle distribution in individual passages, no systematic changes were observed during serial transplantation.

Figure 7 shows the mean transformed Gompertz growth curves of the T61 tumour. The solid lines represent the calculated mean regression lines. The growth is best fitted by two regression lines, one based on the growth data obtained from day 28 to day 44 and the other on the data obtained from day 45 to day 70 after transplantation. An analysis of the growth data showed that proportionality existed among the 'length' (d_1), 'width' (d_2) and 'height' (d_3) of the tumours: $d_3/d_1 = d_3/d_2 = 0.62$. This means that the tumour area is representative of tumour size, and that tumour volume can be calculated from tumour area [24]. The growth curves did not change during serial growth.

It is easy to propagate the T61 tumour in nude mice. After a lag period of about 4 weeks, more than 90% of the tumours demonstrate growth, and the tumour can be propagated equally well in intact or castrated male and female nude mice.

DISCUSSION

The present study confirms previous reports that human tumours serially grown in nude mice preserve many essential characteristics of the donor tissue [1–3]. The human origin of the transplantable T61 tumour was verified by the demonstration of only human chromosomes in the tumour cells (Fig. 1). Ultrastructurally, the tumour cells were found to preserve the characteristics of glandular epithelium, e.g.

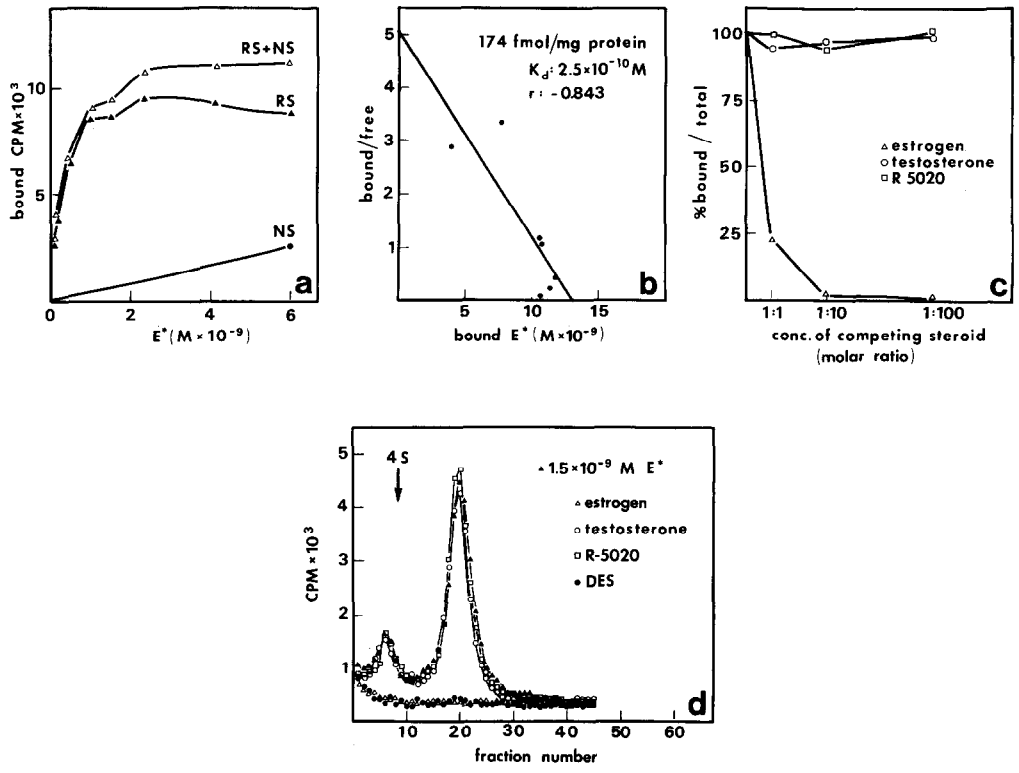

Fig. 4. Oestrogen receptors in the T61 human breast carcinoma grown in a female nude mouse, passage 25. (a) Oestrogen binding to specific (RS) and non-specific (NS) sites. The quantity of receptor-bound steroid (RS) is determined by subtracting NS from the total binding (RS + NS). (b) The specific binding data (RS) from (a) are presented in a Scatchard plot with bound hormone on the abscissa and the ratio of bound to free hormone on the ordinate. The regression curve is calculated by the least square fit from the results in the steepest part of the curve. The intercept with the abscissa gives the binding capacity, whereas the inverse slope of this line gives an approximate apparent value of the dissociation constant of the steroid-receptor complex. (c) The influence of unlabelled oestrogen, testosterone, promegestone (R5020), and DES on the binding of tritiated steroid to tumour cytosol. (d) SDGU analysis of tumour cytosols. Abscissa: fraction number; ordinate: radioactivity recorded (counts/min × 10³).

desmosomes, microvilli, and intracytoplasmic vacuoles (Fig. 3).

The primary patient tumour was classified as a moderately differentiated ductal carcinoma (WHO grade II) (Fig. 2A), whereas the axillary metastasis removed from the patient 3 yr later displayed the characteristics of a medullary carcinoma (Fig. 2B). The morphology of the tumours grown in mice (Fig. 2C) thus more closely resembled the metastasis than it did the primary tumour. One explanation for this difference is that the primary tumour was heterogeneous, containing a dominant cell population with the morphology of an invasive ductal carcinoma, but also a subpopulation of cells with different characteristics, i.e. with the morphology of a medullary carcinoma and with the ability to metastasize and to produce tumours in nude mice. The heterogeneity of the donor tumour may also be the explanation for the finding [26, 27] that primary human breast adenocarcinomas may give rise to tumours with different morphological and/or biological characteristics when transplanted into immuno-suppressed mice.

The chromosome analysis indicated that the T61 tumour is heterogeneous, containing two subpopulations, one with mn 61 and one with mn 122 (Fig. 1). The doubling of the genome and the demonstration of a common marker chromosome (3p-) indicate that the hypertetraploid cells arose by a duplication of the hyperdiploid cells.

Flow cytometric DNA analysis yields information on the DI, which is an important parameter in the identification of cell populations [29], and on the percentage of cells in the cell cycle phases, which is closely related to the proliferative characteristics of the tumour [29]. Further cell kinetic information can be obtained by the sequential analysis of treatment-perturbed tumours [8, 9]. The use of the flow cytometric technique is feasible in tumours grown subcutaneously in nude mice, due to their accessibility for fine-needle aspiration.

The flow cytometric DNA analysis showed only one aneuploid tumour cell population (Fig. 6). A DI of 1.4 indicates that it is the hyperdiploid cell population that is seen in the DNA histograms. It is very likely that a small G_1 peak of

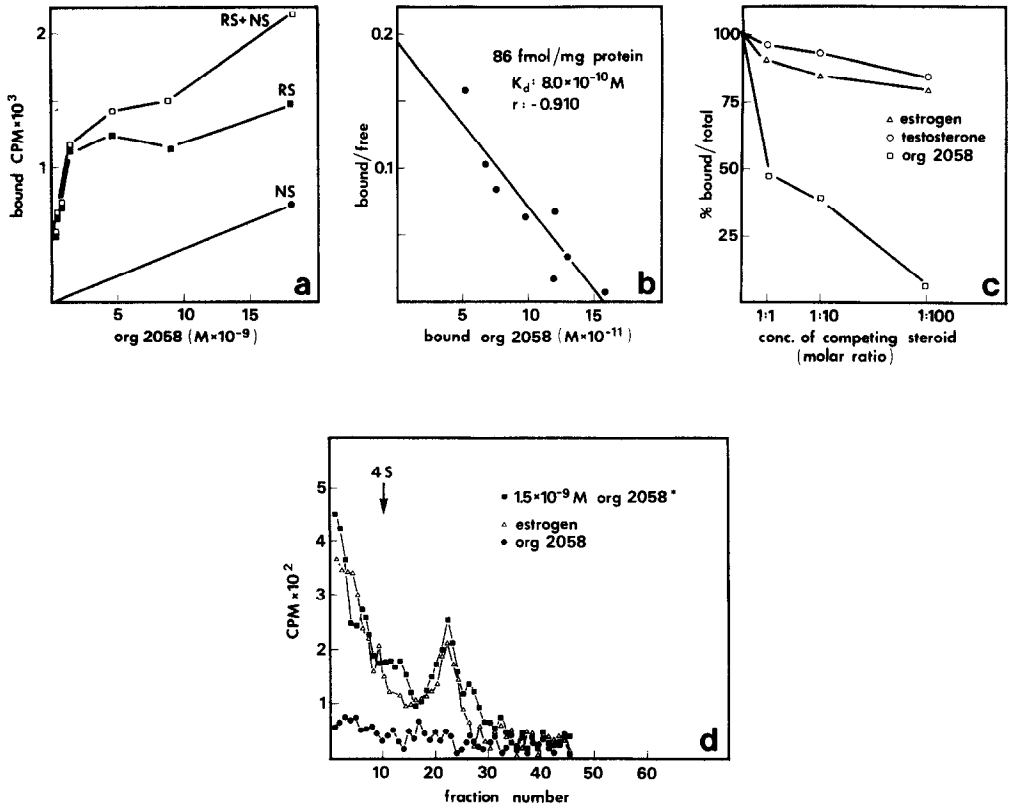

Fig. 5. Progesterone receptors in the T61 human breast carcinoma grown in a female nude mouse, passage 25. (a) Progesterone binding to specific (RS) and non-specific (NS) sites. The quantity of the receptor-bound steroid (RS) is determined by subtracting NS from the total binding (RS + NS). (b) The specific binding data (RS) from (a) are presented in a Scatchard plot with bound hormone on the abscissa and the ratio of bound to free hormone on the ordinate. The regression curve is calculated by the least square fit from the results in the steepest part of the curve. The intercept with the abscissa gives the binding capacity, whereas the inverse slope of this line gives an approximate apparent value of the dissociation constant of the steroid-receptor complex. (c) The influence of unlabeled oestrogen, testosterone, and promegestone (org 2058) on the binding of tritiated steroid to tumour cytosol. (d) SDGU analysis of tumour cytosol. Abscissa: fraction number; ordinate: radioactivity recorded (counts/min × 10²).

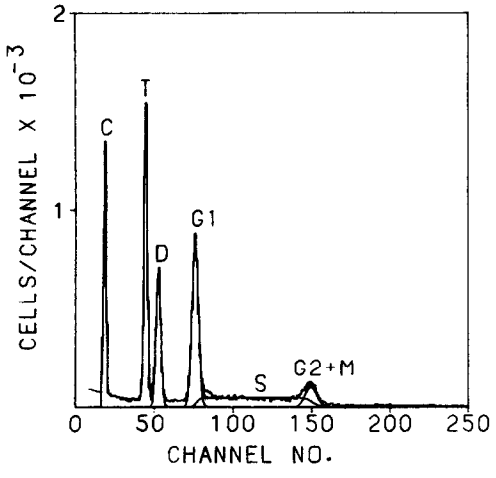

Fig. 6. DNA histogram of a T61 human breast carcinoma grown in a castrated male nude mouse, passage 23. The peak marked D represents diploid mouse stromal cells, and the C and T peaks are internal standards used to calculate the DNA index. The fraction of diploid cells in the tumour was calculated to be 23.08%. The parts of the histogram produced by the tumour cells in G_1, S, and G_2+M are indicated in the figure.

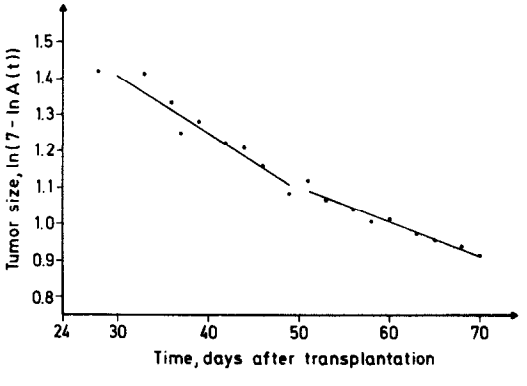

Fig. 7. Mean transformed Gompertz growth curves of the T61 human breast carcinoma grown in castrated male nude mice, passage 18. The points represent mean values of the experimental growth data of 12 tumours. The calculated correlation coefficient of the regression lines are 0.94 (days 28-44) and 0.98 (days 56-70). The α and β values are 0.0149 and 0.095 (days 28-44) and 0.0084 and 0.038 (days 56-70) respectively.

hypertetraploid cells is included in the G_2+M peak of the hyperdiploid cell population since these two peaks coincide. The lack of distinct peaks to the right of the hyperdiploid cell population in the histograms indicates, however, that the hypertetraploid population demonstrated by chromosome analysis constitutes less than 5–10% of the total number of cells in the tumour [28].

The DI of 1.4 of the tumour cells and the DI of 0.98 of the supporting mouse stromal cells (Fig. 6, peak D) permit the separate calculation of the cell cycle distribution of these cell populations. The calculated mean S-phase fraction of the tumour cells is relatively high compared with values hitherto found in clinical breast tumours [30].

A transformed Gompertz function [24] was used for a mathematical description of the tumour growth (Fig. 7) since this transformation depicts the growth rectilinearly, simplifying calculations of relevant tumour growth parameters and the continuous control of tumour growth during serial transplantation. The α and β values (Fig. 7) are within the range found for other hetero-transplanted human tumours [24]. The growth of the T61 tumour is described by two regression lines, as indicated by the change in the slope about 50 days after transplantation (Fig. 7). Since this course of growth is reproducible, the transformed Gompertz growth curves are suitable as a reference in therapeutic experiments [8, 9]. Furthermore, the ability of the T61 tumour to grow equally well in intact and castrated male and female nude mice enables the study of the tumour without the need to give the host hormonal substitutions.

The results of the receptor assay showed that the T61 tumour contained classical oestrogen and progesterone receptors [25]; the receptors in the T61 tumour are thus similar to those found in the MCF-7 [31] and the Br-10 [5] human breast tumours. However, in contrast to the endocrine sensitivity reported for these two other receptor-positive human breast tumours, the growth of the T61 tumour is ovarian independent, and is suppressed by treatment with oestrogen and tamoxifen [8, 9]. It is thus evident that despite similar receptors for oestrogen and progesterone, the T61 tumour represents a different type of receptor-positive breast cancer in terms of endocrine sensitivity, indicating that this tumour may provide a valuable supplement in the study of human breast cancer and endocrine treatment.

Acknowledgements—The technical assistance of Mrs C. Holstein, Mrs V. Hornhaver, Mrs K. Thomsen and Mrs M. Thomsen is gratefully acknowledged. The authors wish to thank Dr J. Rygaard, Kommunehospitalet, Copenhagen, for supplying nude mice, and Mrs Grethe Jensen, Department of Cytology, Frederiksberg Hospital, Copenhagen, for her advice in the preparation of chromosomes.

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