Pergamon

*European Journal of Cancer* Vol. 32A, No. 1, pp. 160–167, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

0959-8049(95)00549-8

## Original Paper

# SR8—The Establishment and Characterisation of a New Ovarian Carcinoma Cell Line and Xenograft Model

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**A new cell line, SR8, and xenograft model of ovarian carcinoma has been established in this laboratory over the past 20 months from a patient with advanced ovarian cancer. Electron microscopic examination of SR8 cells demonstrated the presence of desmosomes and tonofilaments; SR8 cells expressed epithelial membrane antigen (EMA) and glandular associated cytokeratin, all of these confirmed the epithelial origin of this cell line. In addition, SR8 cells expressed CA125, as did the original ovarian tumour. EGF-R and TP53 expression was identified by immunocytochemistry (ICC) in this line. Nearly all the SR8 cells (93%) expressed HLA-class I antigen while 13.5% expressed HLA-DR. SR8 cells showed near-diploid and -triploid chromosome populations with several clonal and non-clonal rearrangements. Subcutaneous and intraperitoneal xenografting of SR8 cells resulted in invasive tumour production at both sites in 3/4 and 4/4 female nude mice, respectively. These xenografts exhibited similar morphology as that of original tumour and were found to express EMA, cytokeratin, CA125 and TP53. The potential research applications of this cell line are discussed.**

**Key words: ovarian carcinoma, cell line, SR8, xenograft, invasion, CA125, EGF-R, TP53**
*Eur J Cancer*, Vol. 32A, No. 1, pp. 160–167, 1996

## INTRODUCTION

OVARIAN CANCER is responsible for 11 000 deaths per year in the U.S.A. and 4000 in the U.K. Despite cytoreductive surgery and newer chemotherapeutic agents, the 5 year survival rate has remained unchanged over the last 25 years. The majority of patients present at stage III or IV. This late presentation limits our understanding of the natural history of this malignancy at the early stages of disease. Through the examination of both *in vitro* established cell lines and *in vivo* animal models of this disease, a better understanding of the cellular mechanisms involved in early tumour establishment and metastatic progression may be obtained, together with the development of newer treatment strategies. *In vitro* investigation and testing is an important part of this research, and in order for this to proceed, the development of tumour cell lines and animal models of the disease are required.

To date, a number of human ovarian carcinoma cell lines have been established [1]. However, a limited number have the capacity to be tumorigenic in nude mice, mimicking the human form of the disease with the production of multiple peritoneal deposits. Monoclonal antibodies currently used to aid diagnosis and determine treatment response rely on tumour cell lines for their development [2–4]. These cell lines have been used to investigate various aspects of tumour biology: the sensitivity of chemotherapeutic substances [5–7]; effects of factors on cellular growth [5, 6, 8]; oncogene expression [9, 10]; and karyotypic abnormalities [5, 6, 11, 12]. However, to our knowledge, no evaluation of an ovarian tumour cell line has incorporated the range of tumour markers that we have examined. The study of cell lines and animal models of the disease may reveal information regarding carcinogenesis and metastasis as well as response to new forms of treatment modalities such as immunotherapy [13] and gene therapy. The more extensive the study of the tumour biological characteristics of an established cell line, with constant reproducible properties, the wider its application for usage within a research setting.

We have established a series of ovarian carcinoma cell lines from the ascites of patients with advanced ovarian cancer, and report on one of these called SR8. This cell line has been studied with respect to cellular morphology, cell growth kinetics, cytogenetics, tumour marker expression, histochemical properties and xenograft formation.

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Revised 17 Jul. 1995; accepted 28 Jul. 1995

## PATIENTS AND METHODS

*Patient data*

An exploratory laparotomy was performed on a 68-year-old woman with ovarian carcinoma FIGO stage IIIc, and a sample of ascitic fluid collected. The histology showed a poorly differentiated mucinous cyst adenocarcinoma of the ovary. The patient was treated with six courses of single agent carboplatin after operation, but her condition deteriorated after initial response to treatment and she died 9 months after initial diagnosis.

*Cell line development*

The cell line was derived from the ascites. Ascitic cells were centrifuged and the cell pellet resuspended in EBSS (Earle's Balanced Salt Solution, ICN Flow Laboratories Ltd, Thame, U.K.). The resultant cell suspension underwent discontinuous percoll (Sigma Chemical Co, Dorset, U.K.) density gradient centrifugation at 800*g* for 30 min [14]. After centrifugation, the upper layer (interface between 30 and 40% percoll containing > 90% tumour cells) was collected and washed. A portion of these tumour cells were cultured with complete culture medium DMEM (Dulbecco's Modification of Eagle's Medium) containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin in 25 $cm^2$ culture flasks (ICN Flow Laboratories). Initially, the cultures grew slowly with the majority of the tumour cells degenerating. Only a few epithelial aggregates were formed that consisted mainly of small rapidly dividing cells and expanded to confluence within 4 weeks. When the culture was passaged, contaminated fibroblasts were first removed by incubation with 0.025% trypsin—0.01% EDTA (ICN Flow) for a short time (< 30 s) followed by shaking of the flasks and pipetting against the surface of the culture. The epithelial cells remaining were then trypsinised and passaged. Fibroblast removal was performed with each passage until no fibroblast cells were visible under inversion microscopy. During the first four passages, the cultured cells were split at ratios of 1 : 1 to 1 : 2 every 2 weeks. Once the cells grew faster and the time to confluence shortened, they were passaged every 7–10 days at a split ratio of 1:3 to 1:5. These cells were designated SR8 and subjected to more than 40 further passages.

A Mycotect kit (Gibco, Midlesex, U.K.) was used to ensure the culture was free from *Mycoplasma* infection at 4 week intervals.

*Population doubling time*

SR8 cells at 32nd passage were placed in 24-well culture plates (ICN Flow) at a density of $2 \times 10^4$ per well in complete medium. The numbers of live cells were then counted in triplicate by Trypan Blue exclusion on days 2, 3, 4, 5, 6, 7, 8 and 10. The growth curves obtained were analysed in order to calculate the doubling time.

*Cytogenetic analysis*

Chromosomes were prepared by standard techniques and G-banded by immersion in 2 × SSC (0.3 M sodium chloride and 0.03 M sodium citrate) at 60°C for 5–10 min, followed by Wrights stain diluted 1:3 in 50% Sorensons buffer (0.06 M $Na_2HPO_4$ and 0.06 M $KH_2PO_4$). Thirty metaphase spreads were counted and those with clear banding fully analysed.

*Fluorescence* in situ *hybridisation*

Fluorescence *in situ* hybridisation (FISH) was performed using chromosome-specific paints (Cambio Ltd, Cambridge, U.K.) and specific probes in order to identify the clonal chromosomal rearrangements observed during karyotypic analysis. One microgramme of each probe was labelled with biotin-14-dATP or digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, Indiana, U.S.A.) using a Bionick kit (Life Technologies Ltd, Paisley, U.K.). DNA was purified by passing through a sephadex G50 column and precipitated with 50 μg salmon sperm DNA and 50 μg *Escherichia coli* tRNA; 20 ng of alpha satellite DNA or 80 ng of labelled probe DNA was mixed with 2–3 μg of Cot-1 DNA (Life Technologies Ltd). Hybridisation was performed as previously described [15]. The chromosome specific paint was used according to the manufacturer's instructions. Images were collected using a cooled CCD camera (Photometrics, with software from Digital Scientific, Cambridge, U.K.) and processed (Adobe Photoshop).

*Probes*

CT4.1 and D1Z2 were used to investigate chromosome band 1p36 [16]. The breakpoint on the long arm of chromosome 17 was analysed using cosmid probes kindly donated by Dr Ellen Solomon (ICRF); RARA, CNP region (17081, 170844, 170335, 171130, F0275, G0775), Gastrin region (A12107, F0631), oestradiol dehydrogenase region (BO3175, D08166), and D17S856, D17S855, C1198, G05149 and G0864. Whole chromosome analysis was achieved using paints specific for chromosomes 1, 3, 6, 17 and X (Cambio Ltd).

*Xenografting*

Athymic female nude mice with ICRF genetic background were used as hosts for xenograft production. Five × $10^6$ SR8 cells at 6th to 10th passage were inoculated subcutaneously into both pectoral regions of four mice, and $10^7$ cells injected intraperitoneally into a further four mice.

*Immunocytochemistry*

A variety of monoclonal and polyclonal antibodies were used to examine formalin-fixed paraffin-embedded tissues of the original tumour, SR8 ovarian carcinoma cells and SR8 xenografts in nude mice using previously described ABC methods [17]. Antibodies to epithelial membrane antigen (EMA), glandular associated cytokeratin, C-erbB2, and antibodies CA125 (to an ovarian tumour marker) and $DO_1$ (to TP53 protein), and epidermal growth factor receptor (EGF-R) were used. However, EGF-R antibody was not used on formalin-fixed paraffin-embedded tissues as these are not stained by this antibody. The sources of the antibodies used and positive control tissues for these antibodies are shown in Table 1. Immunocytochemistry using the above antibodies was performed on SR8 cells grown on chamber slides and cytospin samples. Culture chamber slides were used because this method does not require trypsinisation, a process that may affect cell surface marker molecules. SR8 cells were seeded into chambers slides (Nunc) at a density of $5 \times 10^3$ cells per chamber in culture medium, and cultured for 3 days to allow confluence. Cytospin samples were prepared from SR8 cells harvested from monolayer culture by trypsin–EDTA. Approximately $5 \times 10^3$ cells were used in the cytospin process for each cell-disc. Both chamber slides and cytospin samples were fixed in 1:1 (v/v) methanol–acetone mixture at 4°C for 10 min. Sections of the original tumour and SR8 xenograft undergoing $DO_1$ staining were pretreated using a

*Table* 1. *The description of the antibodies used in ICC and the results from staining of the original tumour, SR8 cells and SR8 xenografts in nude mice*

| Antibodies | Source | Control tissue or cells | SR8 original tumour | SR8 cells | | SR8 xenografts |
|---|---|---|---|---|---|---|
| | | | | Chamber* | Cytospin | |
| EMA (mM)† | Dako | Human breast carcinoma | + | + | ++ | + |
| Glandular associated cytokeratin (mM) | Amersham | Human colon carcinoma | + | + | ++ | + |
| EGF-R (mM) | Sigma | Human squamous carcinoma A431 cells | NT | ++ | ++ | NT |
| CA125 (mM) | Signet | Human ovarian carcinoma | + | ++ | + | + |
| TP53 (mM) | Dr D. Lane | Human pancreas carcinoma cells | – | + | + | + |
| C-erbB2(pR)§ | Dako | Human breast carcinoma | – | – | – | – |

* Chamber, chamber slides. † mM; murine monoclonal antibody; § pR, rabbit polyclonal antibody. ++ Positive staining on more than 50% cells; + positive staining on less than 50% cells; – negative staining. NT = not tested, see text.

microwave protocol as described previously [18]. All the samples tested were treated with 1% hydrogen peroxide in PBS buffer [19] at room temperature for 10 min. After washing in PBS, they were incubated with 2% bovine serum albumin (BSA, Sigma, Dorset, U.K.) for 20 min, followed by application of primary antibodies at an appropriate dilution at 4°C and were left overnight. A secondary biotinylated antispecies antibody was used followed by streptavidin/HRP-labelled-biotin complex (Dako) and then 3,3′-diaminobenzidine (DA, Sigma) as a chromogen.

*Flow cytometric analysis of HLA expression*

Cultured SR8 cells were gently harvested by incubation with 1:5000 versene (Gibco); $0.25–0.5 \times 10^6$ cell aliquots were washed using FACS Flow fluid (Beckton and Dickson). FITC-conjugated monoclonal antibody against HLA-ABC and PE- conjugated monoclonal antibody against HLA-DR were used to quantitatively test MHC antigen expression on SR8 cells. Human lymphocytes from a normal donor were used as positive controls; FITC- and PE-conjugated relevant subclasses of murine immunoglobulin were used as negative controls.

## RESULTS

*Morphology and growth characteristics*

SR8 cells have been maintained in long-term culture for more than 20 months, and passaged over 50 times. They grow as a flat adherent monolayer of mainly polygonal epithelial cells, with occasional multinucleate giant cells (Figure 1). A consistent epithelial morphological phenotype has been demonstrated since this cell line was established. Cultured SR8 cells at electron microscopic examination demonstrate desmosomes and tonofilaments (Figure 2). The doubling time of SR8 cells in monolayer culture was approximately 29 h. This line can therefore be regarded as immortalised as it has been kept in culture for over 300 cell generations.

After the SR8 line was established, an attempt was made to repeat the initial culture of tumour cells using the same ascites specimen, medium and method described previously. This resulted in the growth of morphologically identical epithelial cells as the SR8 cells described. This culture was stopped after the 4th passage and frozen down in liquid nitrogen.

The culture was found to be continuously negative for *Mycoplasma* infection.

**Figure 1. SR8 cells in monolayer culture at early stage after passage showing mainly polygonal epithelial cells forming tumour aggregates (objective × 240).**

*Cytogenetic analysis*

Chromosome counts showed a range of 40–78 chromosomes per metaphase spread. Two populations, one near-diploid and the other near-triploid were present in approximately equal proportions. Four clonal rearrangements and several non-clonal rearrangements were present in both cell populations (Figure 3). The clonal rearrangements included a der(1)t(1;17) (p36?;q21.2?) and a der(6)t(X;6)(q27?;q21?) which were present in 90% and 75% of cells, respectively. A der(X)t(X;6)(q27?;q21?) and a del(3)(p13?) were present in 80 and 75% of the cells, respectively.

*Fluorescence* in situ *hybridisation*

FISH using paints specific for chromosomes 1 and 17 confirmed a non-reciprocal translocation t(1;17)(p36?;q21.2?). Two normal chromosomes 17 were present in every cell and additional chromosome material was present on the derivative chromosome 1. FISH using the probes CT4.1 and D1Z2 showed loss of material from chromosome band 1p36 on the derivative chromosome 1. FISH using the probe RARA (band 17q21.1) showed no signal on der(1). All the other probes from chromosome 17 were present on the der(1) chromosome, localising the breakpoint to between RARA and CNP on chromosome 17 (Figure 4).

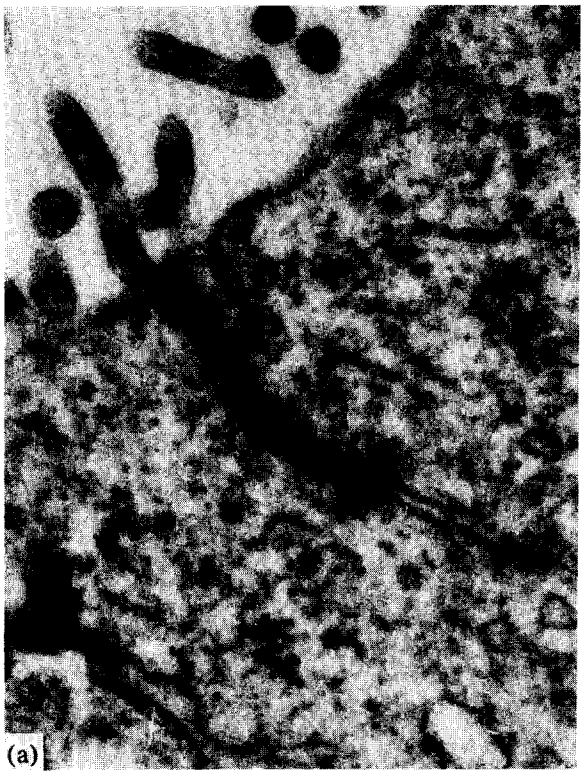


**Figure 2. EM examination of cultured SR8 cells showing (a) desmosomes (D) and (b) tonofilaments (TF). Magnification 6075 × 6.5.**

FISH using paints for chromosomes X and 6 revealed a reciprocal translocation, t(X;6)(q27?;q21?) The exact breakpoints have not yet been determined by FISH analysis.

FISH using paint for chromosome 3 revealed one copy of a normal chromosome 3 and a del(3)(p13?) in every cell. No other chromosome 3 material was detected.

*Xenograft results*

In the group of mice that underwent subcutaneous xenografting, the nodules of subcutaneous tumour were first palpable 2 weeks after initial inoculation. These grew to a mass of 0.5 cm in diameter by 12 weeks, on both pectoral regions in three of the four mice. In the group that underwent intraperitoneal xenografting, the abdominal girth increased from 0.5 to 1.2 cm in all four mice by 8 weeks, but no intraperitoneal tumour nodules could be detected; two of these four mice had approximately 2 ml of ascitic fluid aspirated. These ascitic samples were spun and resultant cell pellets washed in EBSS and seeded into 25 $cm^2$ flasks in the same culture medium as that used above to establish the SR8 cell line. The cultures showed some adherent epithelial cells, but the majority were murine abdominal macrophages. The attempt to culture tumour cells from ascites of these two mice was not successful since the epithelial cells in the cultures underwent degeneration within 2 weeks. However, when the mice were killed at the end of the 12th week after initial inoculation, all four mice in the intraperitoneal xenografting group demonstrated multiple tumour deposits. These were found throughout the abdominal cavity including the liver, diaphragm, mesentery and posterior abdominal walls, and ranged from 1 to 4 mm in diameter.

Subcutaneous xenografts consisted of a multiple nodular mass while intraperitoneal xenografts showed mainly single nodules, and both had a similar granular surface. Subcutaneous and intraperitoneal xenografts exhibited poorly differentiated adenocarcinoma morphology with frequent mitotic figures which were similar to the original ovarian tumour. There were a few foci of glandular differentiation within the xenografts, and as in the original tumour, mucin production was demonstrated. The subcutaneous xenografts demonstrated invasion into the host muscle. The intraperitoneal tumour was found to invade through the liver capsule directly into the liver parenchyma (Figure 5).

*Immunocytochemistry and flow cytometry*

The immunocytochemical results are shown in Table 1 and Figures 6 and 7. The SR8 cellular preparations demonstrated positive staining for EMA, glandular associated cytokeratin, EGF-R and CA125 on both chamber slide cultured cells and cytospin samples. The original ovarian tumour and SR8 xenografts demonstrated positive staining for EMA, cytokeratin and CA125. The original tumour was negative for TP53, whilst both SR8 cells and xenografts showed positive TP53 staining. None of the samples stained for C-erbB2.

The HLA expression of SR8 cells as detected by flow cytometry demonstrated positive expression of HLA-DR and -ABC at rates of 13.5 and 93% respectively.

## DISCUSSION

This study describes a newly established ovarian carcinoma cell line, designated SR8, which was derived from the ascites of a patient with a poorly differentiated ovarian mucinous cystadenocarcinoma. The cell line was confirmed as epithelial in origin on the basis of electron microscopic detection of desmosomes and tonofilaments and positive immunocytoch-

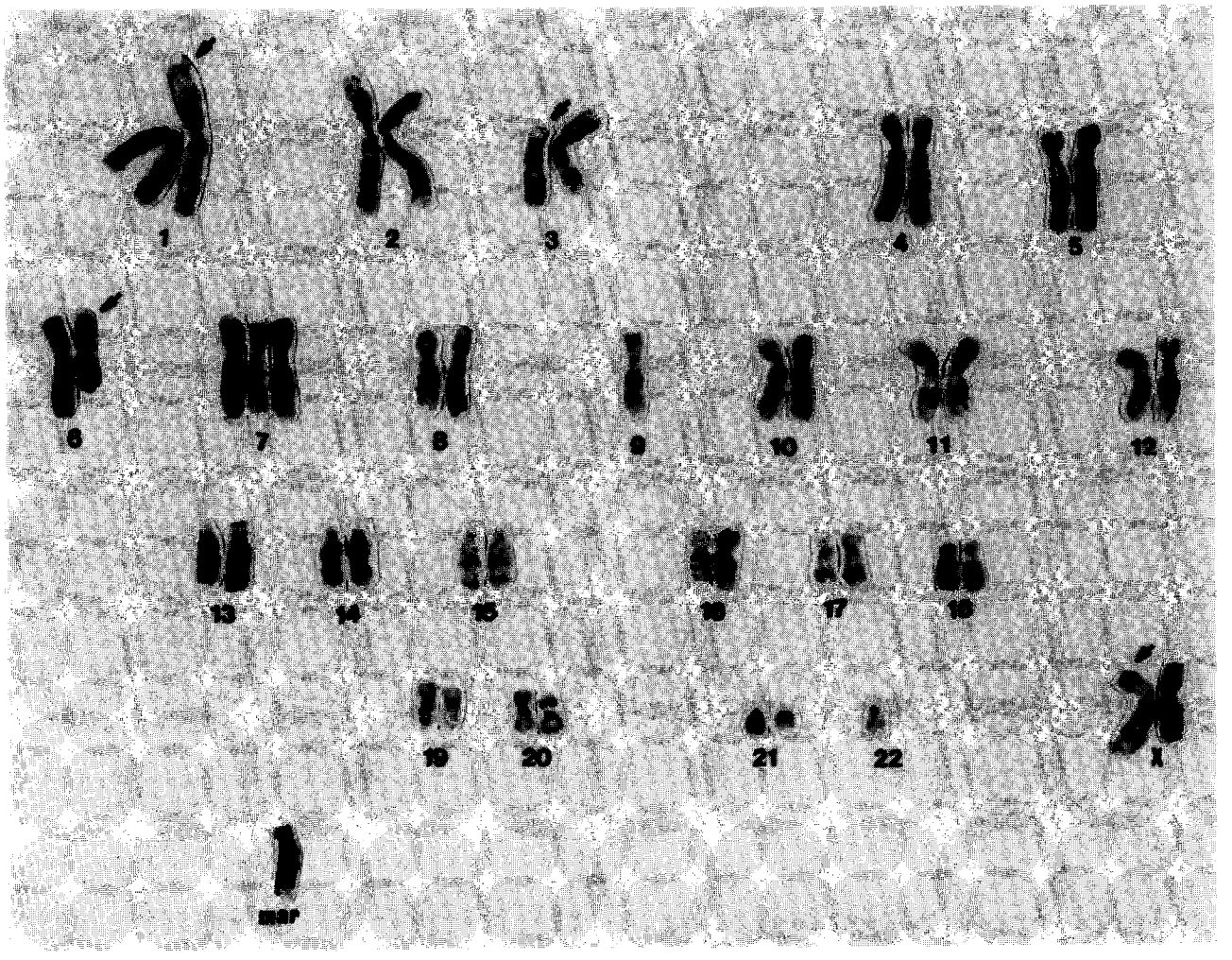


**Figure 3. Representative karyotype showing four clonal chromosome rearrangements (arrowed) and one non-clonal rearrangement (mar).**

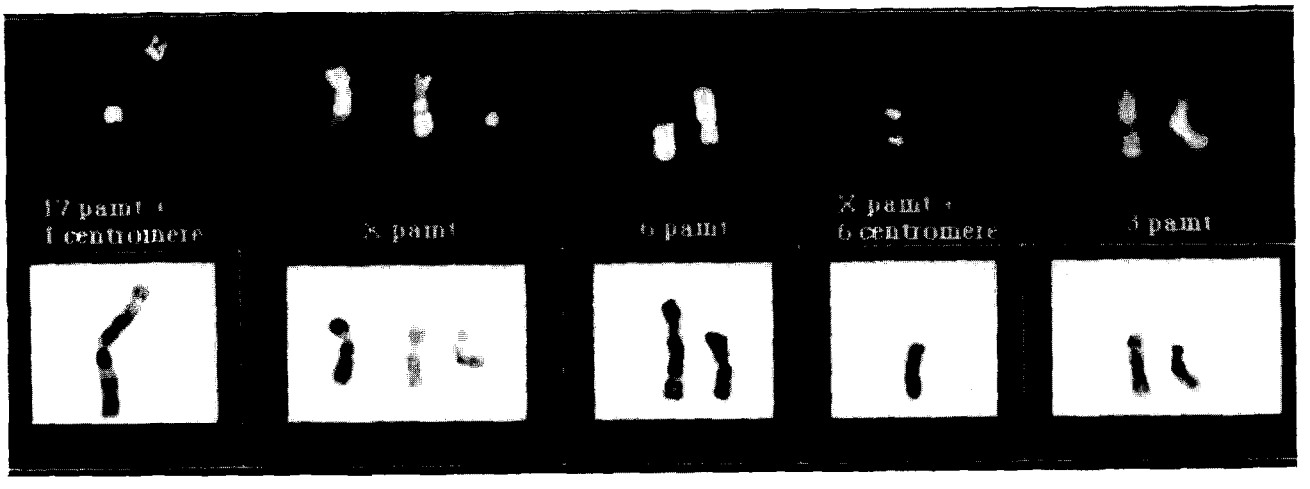


**Figure 4. Partial karyotypes showing FISH results with different chromosome paints and the corresponding G-banded chromosomes.**

**Figure 5. SR8 intraperitoneal xenograft tumour deposit (T) in nude mouse showing invasion (Ti) into host liver tissue (L) (objective × 200).**

emistry for EMA and glandular associated cytokeratin. SR8 cells produced both subcutaneous and intraperitoneal tumour deposits after inoculation into female nude mice. The xenografts exhibited mucin production and a similar morphology to that of the original tumour. They also stained positive for several human cellular markers such as EMA, cytokeratin, CA125 and TP53, confirming their human origin.

Several ovarian carcinoma cell lines have been established in the past. However, only a few have demonstrated tumorigenic capacity in nude mice, in particular, the ability to develop intraperitoneal xenografts that mimic the human form of this disease. In recent years, tumour marker expression on newly established ovarian carcinoma cell lines and their xenografts has been investigated. For instance, the UCI 101 line was found to express several cellular markers, including EMA, cytokeratin, CA125 and EGF-R [20]. In addition, high levels of CA125 were detected in the serum of mice bearing intraperitoneal xenografts developed from UCI 101 cells. Langdon and colleagues reported that the PE04 cell line was oestrogen receptor (ER) positive. When xenografted, the PE04 cells continued to express ER as well as progesterone receptors

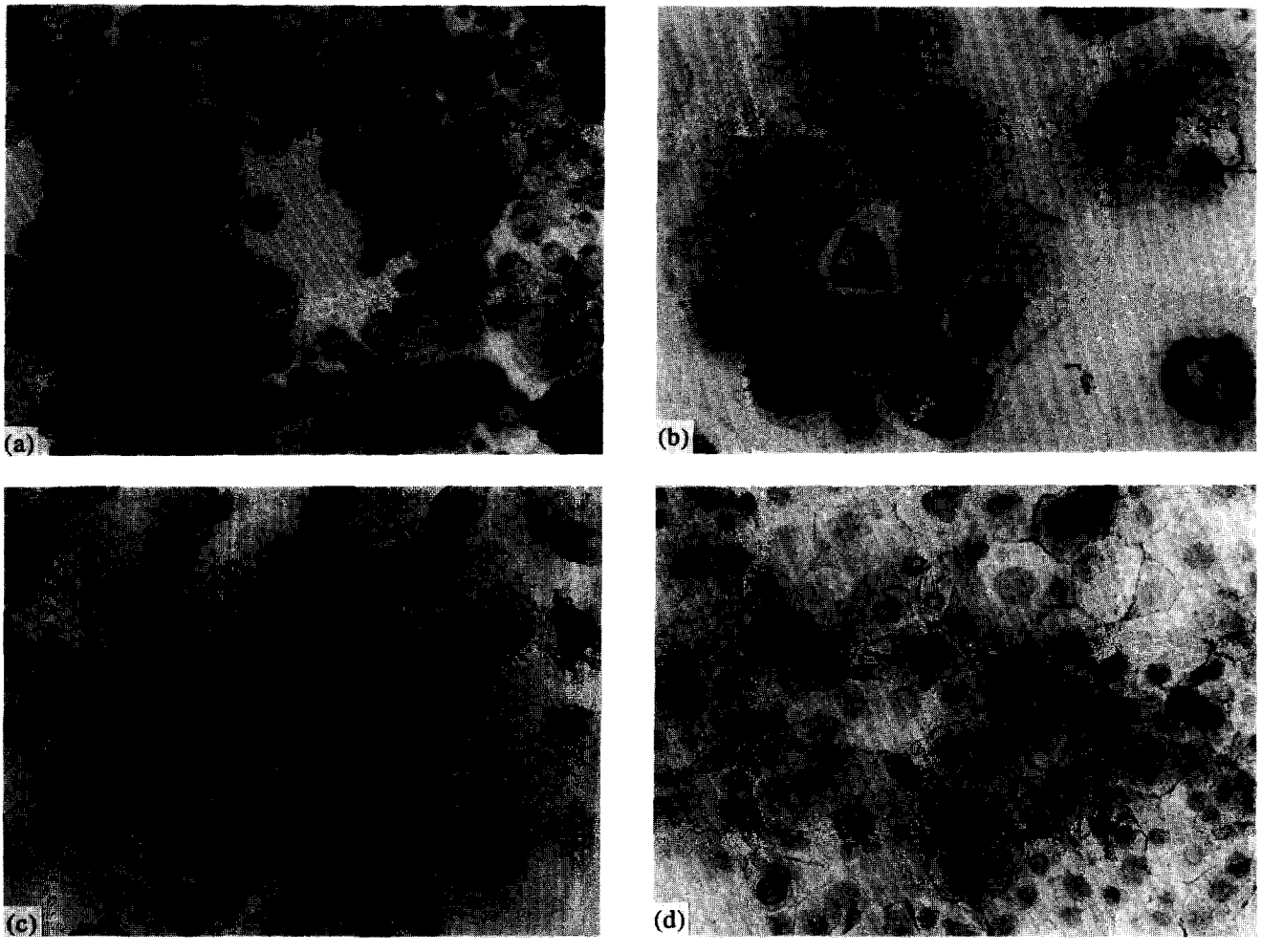


**Figure 6. Immunohistochemical staining for SR8 cells, cytospin samples stained for (a) EMA and (b) glandular associated cytokeratin; and chamber slide samples stained for (c) EGF-R and (d) CA125 (objective × 200).**

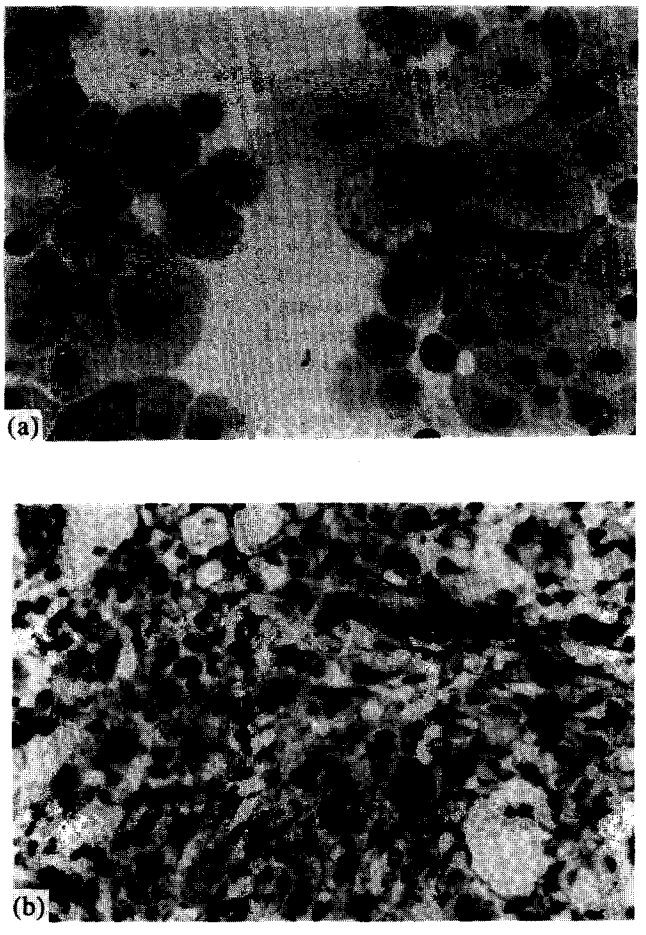


**Figure 7. Immunohistochemical staining for TP53 on (a) cytospin samples of SR8 cells and (b) the SR8 xenograft (objective × 200).**

(PR); indicating that ovarian cancer may be hormone-sensitive and potentially responsive to endocrine therapy [21].

Preparations of the SR8 cell line in both cytospin samples and chamber slides showed positive immunocytochemical staining for EGF-R and TP53, as well as EMA, cytokeratin and CA125. The SR8 original tumour and SR8 xenografts were positive for EMA, cytokeratin and CA125. However, the xenografts also stained positive for TP53.

Aberrations of the tumour suppressor gene *TP53* have been demonstrated in many human malignancies, including ovarian carcinoma. Yaginuma and associates [22] showed altered levels of *TP53* gene expression and/or mutant forms of the *TP53* gene in six ovarian carcinoma cell lines. Frank and colleagues [23] reported that immunoreactivity for TP53 correlated closely with the presence of mutation in the *TP53* gene, and demonstrated a significant correlation between *TP53* overexpression and allelic loss in ovarian carcinoma. There are several possible explanations for positive staining for TP53 in the cell line but not in the original tumour. The cell line was developed from metastatic tumour cells in the ascitic fluid which may represent a different population from those within the solid ovarian tumour. Alternatively, the cell line may represent a small cellular component of the original tumour which was selected during *in vitro* culture becoming the dominant cell type in the SR8 line. Another explanation is that the cell line may have developed a *TP53* mutation or abnormal *TP53* expression during *in vitro* culture. We are currently examining the *TP53* gene in the SR8 cell line for mutation.

Cytogenetic analysis of the SR8 cell line showed several interesting chromosome aberrations, including a clonal rearranged chromosome, der(1)t(1;17)(p.36?;q21.2?), which was present in 90% of cells. This chromosome is derived from an unbalanced 1;17 translocation, resulting in loss of genetic material from band 1p36 and gain of material from the long arm of chromosome 17. Translocations involving band 1p36 have been noted previously in primary ovarian carcinomas [reviewed in ref. 24], although the partner chromosomes were not identified in most cases. It may be significant that a similar der(1)t(1;17) chromosome has been noted in neuroblastoma [25]. Although the breakpoints in the neuroblastoma der(1)t(1;17) chromosome are heterogeneous, they also result in net loss of 1p36 material and gain of 17q material [26].

Loss of heterozygosity (LOH) on both arms of chromosome 17 has been described in ovarian carcinoma by several groups, indicating the involvement of tumour suppressor genes besides *TP53* in ovarian tumorigenesis [27]. The presence of two apparently intact copies of chromosome 17 in addition to the segment on the der(1) chromosome does not preclude the possibility that deletion of chromosome 17 material is important in SR8. The translocation which generated the der(1) chromosome may have occurred first, deleting one or more tumour suppressor genes on chromosome 17. The intact copy of chromosome 17 which could have inactivating mutations of these tumour suppressor genes, may have duplicated subsequently. Interestingly, the *BRCA1* gene is mutated in sporadic ovarian tumours [28]. However, because this gene is retained on the der(1) chromosome, at least two inactivating mutations (or deletions) would have had to occur if it was involved in SR8 tumorigenesis.

Other cytogenetic aberrations in the SR8 cell line involve chromosomes 3, 6 and X. Rearrangements of these chromosomes, predominantly involving deletions or loss of heterozygosity, have been noted before in primary ovarian tumours and in cell lines [24]. The derivative chromosomes 6 and X in SR8 appear to be the product of a reciprocal translocation, t(X;6)(q27?;q21?). FISH analysis confirms that the del(3)(p13?) leads to loss of chromosome 3 material from the genome. Chromosome transfer studies have recently identified three regions of chromosome 3 which may contain tumour suppressor genes involved in ovarian tumorigenesis [29]. Two of these regions are located in bands 3p24.2–23 and the third in 3p21.2–21.1. These regions are all lost from the del(3)(p13?) chromosome in SR8.

In summary, we have developed a stable ovarian carcinoma cell line which expresses a variety of tumour markers and has chromosome rearrangements which are similar to those found in primary tumours. The cell line is capable of producing an intraperitoneal model of disease resulting in tumour xenografts with similar tumour morphology and receptor expression as the original tumour. The cell line may be useful as a model for further studies concerning tumour biology, evaluation of cytokine effects on the control of tumour growth, new drug treatments and future studies of gene therapy. SR8 also provides a source of continuously growing tumour cells bearing various tumour cell markers for the development of new immunodiagnostic techniques.

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**Acknowledgements**—This research was supported by a grant from the Special Trustees of St Thomas's Hospital, Lambeth Palace Rd, London SE1 7EH, U.K., and by the Imperial Cancer Research Fund. Probes were kindly donated by Dr E. Solomon, Imperial Cancer Research Fund.