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Transplantation of a human ovarian cystadenocarcinoma into severe combined immunodeficient (SCID) mice – formation of metastases without significant alteration of the tumour cell phenotype

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Summary. Human ovarian papillary cystadenocarcinoma cells were injected intraperitoneally into severe combined immunodeficient (SCID) mice. After intraperitoneal application the cells, designated SoTü, grew well *in vivo*, lodged on to the peritoneum, formed local metastatic deposits, led to the development of ascites in the mice and formed distant metastases in the lungs. If lodged in the ovary, the morphology of the SoTü tumour remarkably resembled that of the primary tumour in the patient. In contrast, several attempts failed to maintain the SoTü cells *in vitro*. If SCID mouse ascites derived SoTü were transplanted subcutaneously in SCID mice, they formed cystic tumours which also metastasized into the lungs. Immunophenotypical analysis of cell adhesion molecule expression, cell proliferation markers, various oncoproteins, keratin, vimentin, and lectin binding site expression all showed striking similarity between the primary tumour and the SCID mouse explants. In particular, expression of binding sites for the lectin *Helix pomatia* agglutinin (HPA), which has been shown to be an index of metastatic potential in several human carcinomas, was found on the primary tumour as well as on tumour cells grown in SCID mice, indicating that HPA might be a prognostic indicator in ovarian carcinoma as well. Our results demonstrate that the human/SCID mouse system can mimic growth and distant metastasis formation of human ovarian carcinoma. Although the formation of distant metastases is a relatively rare event in patients, this model system might help to elucidate mechanisms of metastasis formation in ovarian cancer.

Keywords: cell adhesion molecules, cell proliferation marker, electron microscopy, *Helix pomatia* agglutinin, metastasis, ovarian cancer, orthotopic transplantation, severe combined immunodeficient mice, tumour marker

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Ovarian cancer has an overall bad prognosis and therefore models allowing a better understanding of the biological behaviour of the tumour and the development of new therapeutic strategies are needed. Initially, transplantable murine ovarian cancer models have been used for this purpose (Frei 1982; Venditti 1983). However, greater relevance to the human situation can be obtained by using cells from human ovarian carcinomas *in vitro* (Wilson 1984; Hills *et al.* 1989) and *in vivo* by transplanting human ovarian cancer cell lines into the peritoneal cavity of nude mice (Malik *et al.* 1991) or by transplanting samples of human ovarian carcinomas orthotopically (Fu & Hoffman 1993; Astoul *et al.* 1993). The use of a human/animal interface is of particular interest since the complexity of the metastatic process is not completely modelled *in vitro* (Williams *et al.* 1993).

The establishment of a metastatic human papillary ovarian cystadenocarcinoma in SCID mice is reported in this paper. In addition, immunophenotypical analysis of a variety of cellular markers related to prognosis was performed on the primary tumour, the malignant effusion in the SCID mice and on the explanted tumours grown subcutaneously in the SCID mice.

Patient and methods

Clinical data

The patient was a 54-year-old post-menopausal gravida 10 obese woman who presented with acute abdominal pain. Physical examination revealed a left ovarian mass. Chest X-ray and CT showed no evidence of a primary or secondary tumour. At laparotomy a left ovarian tumour measuring up to 15 $\times$ 10 $\times$ 5 cm was removed by unilateral salpingo-oophorectomy. The right ovary was normal and no signs of peritoneal carcinomatosis or lymph node involvement were detected. Histology revealed a moderately differentiated serous papillary cystadenocarcinoma of the ovary (stage FIGO IC). Post-operatively the patient received four courses of combined chemotherapy consisting of cisplatin, treosulfan and cyclophosphamide. Chemotherapy was discontinued because of the progression of the disease which presented as massive ascites due to peritoneal carcinomatosis. Several taps were performed to relieve ascites; cytological examination showed the presence of malignant cells. The patient died 14 months after diagnosis.

SCID mouse experiments

Ascites fluid (45 ml) from one of the therapeutically indicated taps was sent by express mail to the UK and used for the following experiments. The malignant effusion was spun down at 700*g* for 5 minutes and the cell pellet was immediately injected intraperitoneally into a female SCID mouse. After 12 weeks the development of ascites was visible and the mouse was sacrificed. The SoTü cells were pelleted as above and one part of the pellet was injected into three female SCID mice which were sacrificed 6 weeks later. The second part was fixed in Karnovsky's glutaraldehyde and formaldehyde fixative for 3 h at 4°C.

Following the 8th passage, 0.5×10^7 cells were injected subcutaneously in the region between the scapulae of SCID mice. After 6 weeks the mice were sacrificed and the primary tumours grown on the back and lungs were fixed in neutral buffered formalin and processed for wax histology.

Cell culture

After the first passage in the SCID mouse SoTü cells were grown *in vitro* using several different types of media supplemented with 10% fetal calf serum, 1% penicillin and streptomycin and 1% fungizone solution. The cells were kept in a humidified 5% CO_2 atmosphere. The following media (and their supplements) were used: McCoys 5 A (+1% L-glutamine), Eagles MEM (+ 1% non-essential amino acids, 1% 100 mM sodium pyruvate, 1% L-glutamine), RPMI 1640 (+1% HB supplement consisting of 1320 mg oxalacetic acid, 80 mg bovine insulin, 550 mg sodium pyruvate dissolved in 100 ml distilled H_2O), DMEM (+1% HB supplement) and L15 (+1% HB supplement), the latter being CO_2 free.

Histology and histochemistry

For electron microscopy, the tissue was washed overnight in 0.1 M phosphate buffer (pH 7.4) after fixation and was post-fixed for 1 hour in aqueous 1% OsO_4 and afterwards embedded in Araldite. Semi-thin sections were cut on a Reichert Ultracut E ultramicrotome and 1-μm sections were stained with 1% toluidine blue in 1% borax on a hot plate. For electron microscopy the sections were stained with 1% aqueous uranyl acetate for 30 minutes and with Reynolds' lead citrate for 15 minutes. Photographs were taken on a Philips EM 300 transmission electron microscope. The third part of the sample was fixed in neutral buffered formalin and processed for routine wax histology.

Haematoxylin and eosin stain and periodic acid Schiff reaction (PAS) with/without diastase pretreatment were carried out on the primary tumour and on the cells grown in the ascites from the first, fourth and sixth passage

Table 1. Lectins used in this study, their abbreviation and nominal sugar specificities

Lectin	Abbreviation	Sugar specificity
Griffonia simplicifolia	GSA-I	*N*-acetylgalactosamine
Vicia villosa	VVA	*N*-acetylgalactosamine
Helix pomatia	HPA	*N*-acetylgalactosamine
Peanut	PNA	Galactose
Concanavalin A	Con A	Mannose, glucose
Lens culinaris	LCA	Mannose, glucose
Ulex europaeus	UEA-I	Fucose
Wheat germ	WGA	*N*-acetylglucosamine
Phaseolus vulgaris	PHA-E	Complex carbohydrates
Phaseolus vulgaris	PHA-L	of *N*-acetyllactosamine type

through SCID mice as well as from the subcutaneous tumour implants. In addition, immunohistochemical analysis was carried out using the following antibodies: KL1 (anti-pancytokeratin, Dako, Hamburg, FRG), vim (anti-vimentin, Dako, Hamburg, FRG), anti-CA125 (IS, Dreieich, FRG), VVF7 (anti-CD44v6, Bender & Co, Vienna, Austria), anti-E-cadherin (Takahara, Shiga, Japan), MIB-1 (Dianova, Hamburg, FRG), PC10 (anti-proliferating cell nuclear antigen PCNA, Dako, Hamburg, FRG) and DO7 (anti-p53, Dako, Hamburg, FRG). Biotin avidin complexes (ABC) with horse-radish peroxidase as a marker enzyme and diaminobenzidine were used for visualization of the binding sites (Hsu *et al.* 1981). Lectin histochemical analysis of the carbohydrate residues was performed using biotinylated lectins (10μg lectin/ml) without prior trypsinization (for lectins used, their abbreviations and sugar specificity see Table 1; for methodological details see Schumacher *et al.* 1995b). Control experiments using 0.3 M of the appropriate haptenic sugars (the same monosaccharides as the sugar specificity of the lectins in Table 1 except for PHA-L, -E where 10 μg/ml thyroglobulin was used) were performed. The binding of HPA was assessed by an indirect immunoperoxidase technique (Schumacher *et al.* 1995a).

Results

Inoculation into SCID mice and cell culture experiments

Twelve weeks after primary inoculation into the peritoneal cavity of a female SCID mouse a visible ascites had developed. SoTü ovarian cancer cells could be demonstrated in the malignant effusion which was almost always haemorrhagic. The SoTü cells were serially transplanted into the peritoneal cavity over a period of 2 years to other SCID mice. In general, 4–6 weeks after inoculation malignant effusion developed in these mice. Six weeks after inoculation of SoTü cells into the subcutaneous tissue on the back of the SCID mice, solid tumours measuring up to 15 $\times$ 10 $\times$ 10 mm developed.

Attempts to cultivate the SoTü cells under standard cell culture conditions using the above mentioned defined tissue culture media were performed from all passages; however, after five or six passages in cell culture the cells did not proliferate and eventually died.

Morphological findings

The original patient tumour represented a typical serous papillary cystadenocarcinoma (grading 2) of the ovary growing on a fibrovascular stroma (Figure 1 A), sometimes with central necrotic parts. The same growth pattern was seen in those tumours which had engrafted in or near the ovary after intraperitoneal inoculation (Figure 1B). The tumours developing in the back of the SCID mice also presented as cystic mass lesions with central necrosis (Figure 1C); however, the lumina of the cysts were smaller and appeared to be more densely packed than those of the primary tumour. The histological examination of the lungs of those animals which had been transplanted with SoTü cells intraperitoneally or on the back revealed the presence of disseminated (micro)-metastases (Figure 1D). These metastases were much more frequently observed after intraperitoneal injection than after subcutaneous injection.

The SoTü cells grown in the ascites of all passages could be found either as single cells or as small cohesive aggregates (Figure 2 A). The cytoplasm of the cells grown on the back of SCID mice were PAS negative. In contrast the tumour cells grown in the ascites showed a strongly positive cytoplasmic PAS reaction which was markedly reduced after diastase pretreatment.

In semi-thin sections SoTü cells transplanted onto the back of SCID mice grew intravascularly and no distinct

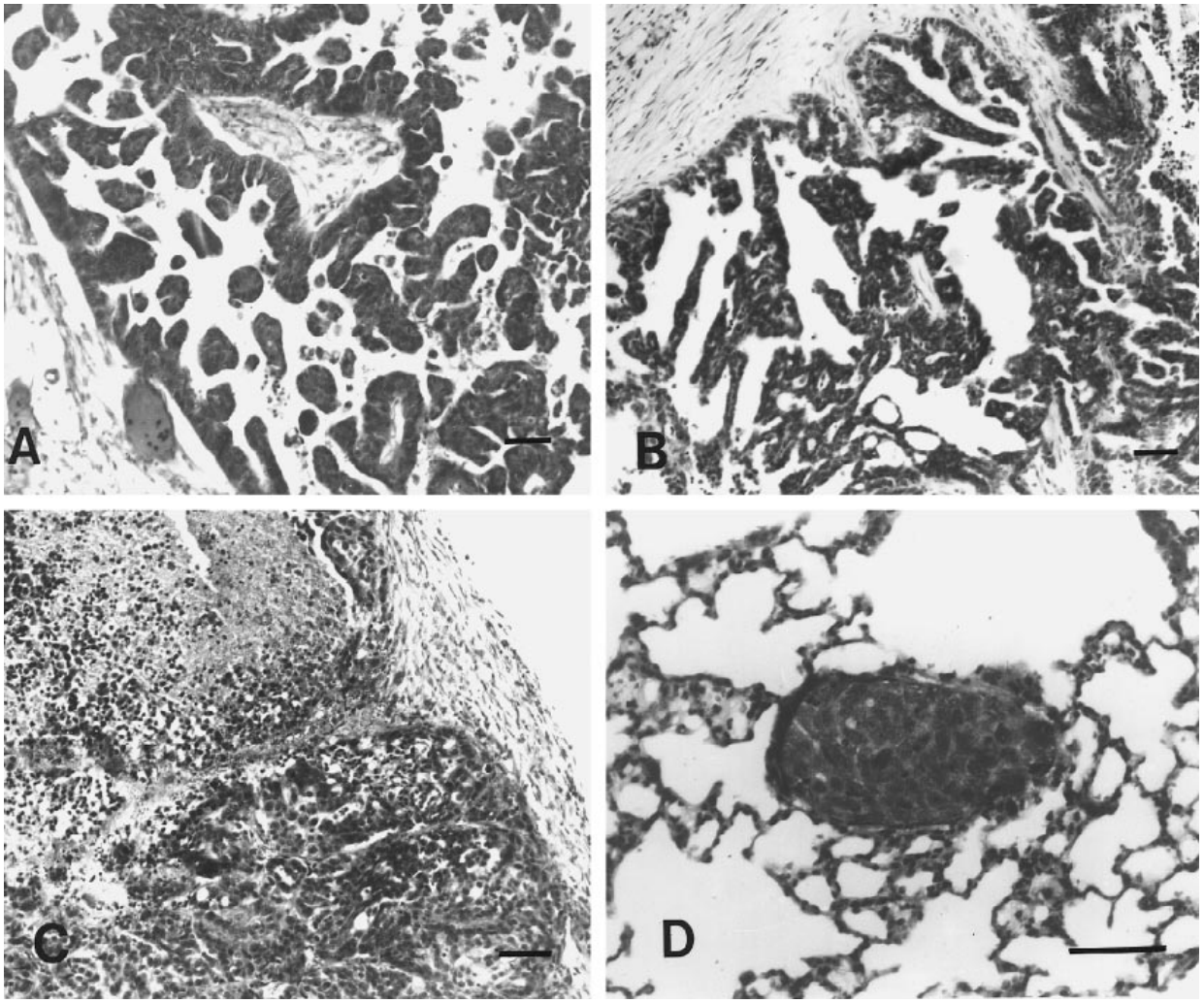

Figure 1. A, Morphology of the original patient tumour: serous papillary cystadenocarcinoma of the ovary, HE (bar 50 μm). B, The same tumour but grown from SoTü tumour cells from the peritoneal tap from this patient in SCID mouse pelvis adjacent to the ovary. Note the striking similarity of A, HE (bar 50 μm). C, The same SoTü tumour cells but grown after subcutaneous injection on the back of a SCID mouse. The principal morphology of the tumour is similar to A and B but the cysts are much smaller, HE (bar 50 μm). D, Lung metastasis of SoTü cells after inoculation on the back of a SCID mouse, HE (bar 50 μm).

basement membrane of tumour cell aggregates grown as cysts could be identified. In electron microscopy the SoTü cells grown intraperitoneally in suspension showed a polarization typical for epithelial cells with formation of microvilli at the apical membrane. If grown in aggregates the SoTü cells were connected via desmosomes (Figure2 A) and contained abundant glycogen granules. The nuclei were often indented and irregular in shape but otherwise the SoTü cells contained the normal set of organelles and only the presence of numerous elongated mitochondria was remarkable. If SoTü cells grew as solid tumours on the back of SCID mice, the tumour cells grew without the formation of a basement membrane, but microvilli bordered the region of the cells exposed to the extracellular matrix (Figure 2B).

The findings of the light microscopic histo and cytochemical analyses are summarized in Table 2. In general the immunohistochemical findings indicated that the immunoreactivity in the original patient's tumour and in the explanted tumours in the mice was similar. The widely used tumour marker for ovarian cancer, CA 125, showed a stable expression in the different samples investigated (Figure 3). E-cadherin not only stained the primary tumours, but also labelled metastases. In general, the E-cadherin immunoreactivity was much more pronounced in the larger metastases than in the smaller

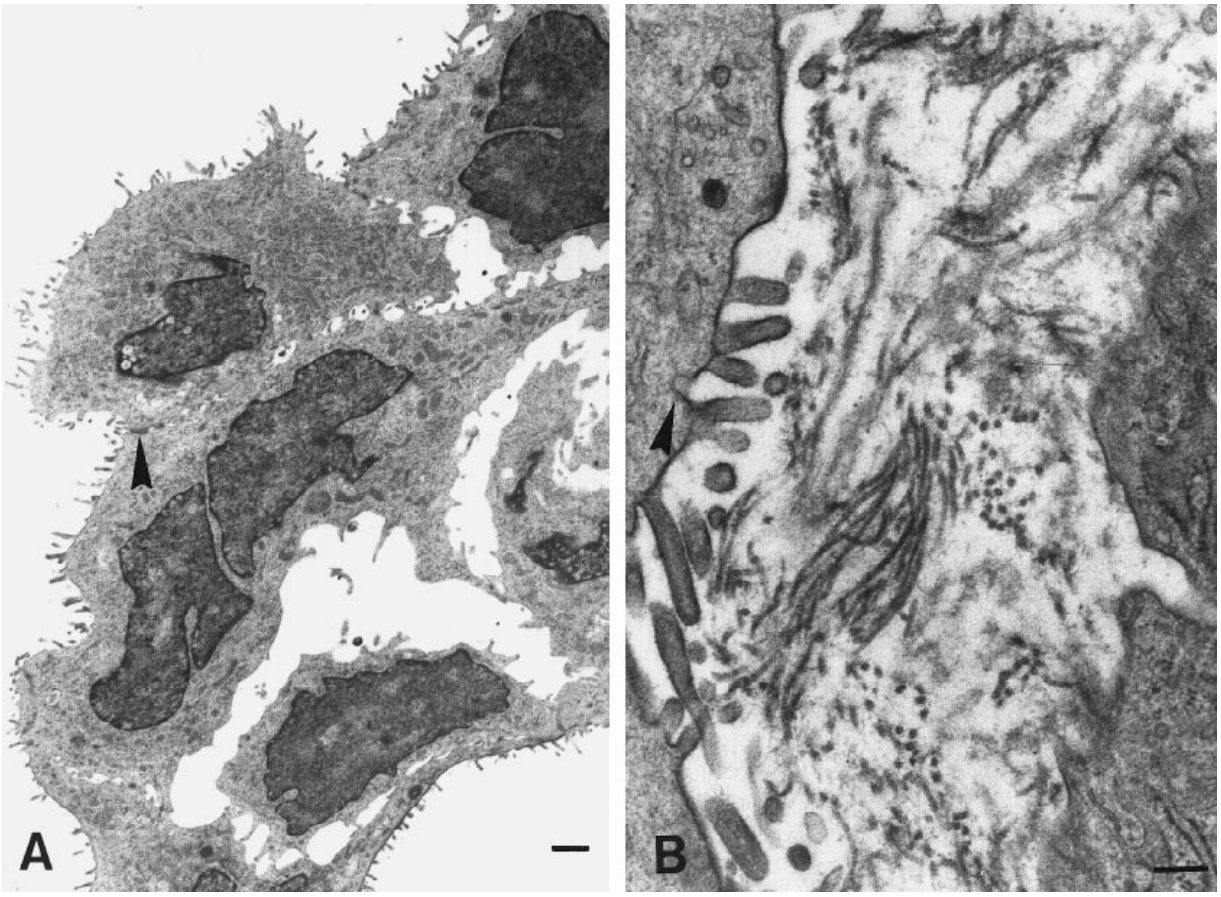

Figure 2. A, Electron micrograph of a SoTü tumour cell aggregate after the first intraperitoneal passage in SCID mice. Note the polarity of the cells with the formation of microvilli at the apical surface and the formation of desmosomes between adjacent tumour cells (bar 1 μm). B, SoTü cells grown as solid tumours (bar 0.2 μm). Note the presence of microvilli at the tumour-stroma interface and the pit at the cell membrane (arrowhead) indicating either a secretory or an endocytotic activity.

Table 2. Summary of the immunochemical and lectin histo and cyto-chemical results. The numbers refer to the percentage of tumour cells stained

			Passage in SCID ascites	
Marker	Primary	Solid SCID	1st	6th
KL1	100	90	90	40
VIM	10	10	0	10
CA 125	90	90	100	100
CD44v6	40	<10	15	<10
E-CAD	70	100	90	100
MIB 1	40	60	30	30
PCNA	80	100	85	40
p53	50	100	90	40
GSA-I	90	20	90	80
VVA	100	20	80	80
PNA	100	30	90	90
Con A	100	80	80	80
LCA	100	40	100	100
UEA-I	100	0	0	0
WGA	100	>90	100	100
PHA-E	100	40	100	100
PHA-L	100	0	20	0

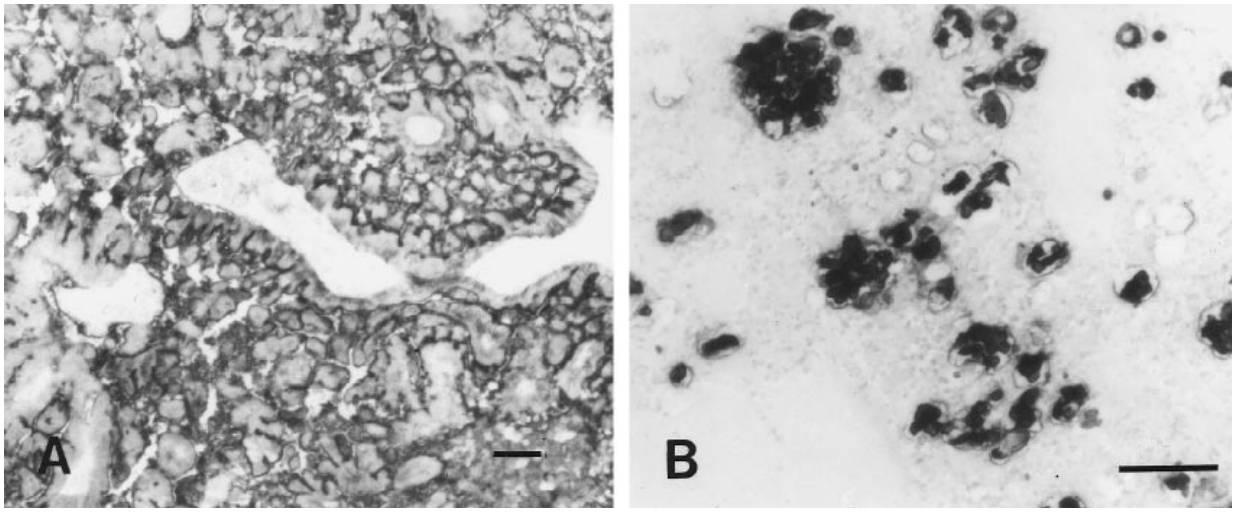

Figure 3. A, The tumour marker CA 125 is particularly expressed at the cell surface of the patient's tumour (bar 50 μm) and B, its expression is maintained in both single and aggregated SoTü cells grown in SCID mouse ascites (B, bar 50 μm).

ones. Cytokeratin staining was observed in all samples assuring in principle the epithelial nature of the tumour cells grown in SCID mice. However, the expression of cytokeratin positive cells within the different tumour cell populations varied, indicating a possible dedifferentiation with respect to this marker during passaging.

A relatively uniform lectin binding pattern was observed in the primary tumour and with all the explanted tumours. Usually the whole cytoplasm of tumour cells was stained, often with a pronounced staining of the (apical) membrane. Intensive HPA binding could be observed in all samples (primary tumour, ascites, subcutaneous tumour, lung metastases; see Figure 4); however, the binding was more intense in the ascites grown cells than in the cells from the solid tumours. While differences in lectin binding patterns between the primary tumour and the first SCID mouse passage were limited to VVA, UEA-I, PHA-E and PHA-L, more pronounced differences in lectin binding patterns were observed between the primary tumour and the sixth passage. Comparing the results of the lectin staining patterns between the fourth and sixth passage indicated that, with a few exceptions, the carbohydrate expression of the tumour cells had stabilized (data not shown). In general, the control experiments using the inhibitory sugars resulted in complete inhibition of lectin binding. However, WGA retained an apical staining of the tumour cells after pre-incubation with the inhibitory sugar and LCA showed an overall decreased lectin reactivity after pre-incubation with the inhibitory sugar.

Discussion

The present study has demonstrated the feasibility of transferring human ovarian cystadenocarcinoma cells into the peritoneal cavity of SCID mice. It was possible to transplant serially the SoTü tumour cells which were derived from a malignant ascites in these mice while several attempts to cultivate the tumour cells *in vitro* using a variety of tissue culture media and supplements were unsuccessful. These findings indicate that the SCID mouse is superior to cell culture in establishing this particular SoTü human ovarian cancer cell ex-patient and, in accordance with earlier observations, shows that SCID mice are capable of propagating human tumours *in vivo* which have not previously been grown *in vivo* (Williams *et al.* 1993). In one study a human ovarian carcinoma was successfully engrafted only in SCID mice and not in any other strain of immunodeficient mice (Garofalo *et al.* 1993).

The ultrastructural features of SoTü cells are consistent with those of a tumour of mixed histological type as defined by Fenoglio (1980) and their ultrastructural morphology is similar to those described for other cell lines where the presence of glycogen was noted, but only found in those cells grown in suspension and not in the solidly grown tumours (Hills *et al.* 1989). The accumulation of glycogen in cells growing in suspension is not a new observation. Walter (1995) noted the accumulation of glycogen in bovine oviduct epithelial cells grown in suspension culture, whereas the cells grown *in situ* did not contain glycogen granules. Two possible explanations

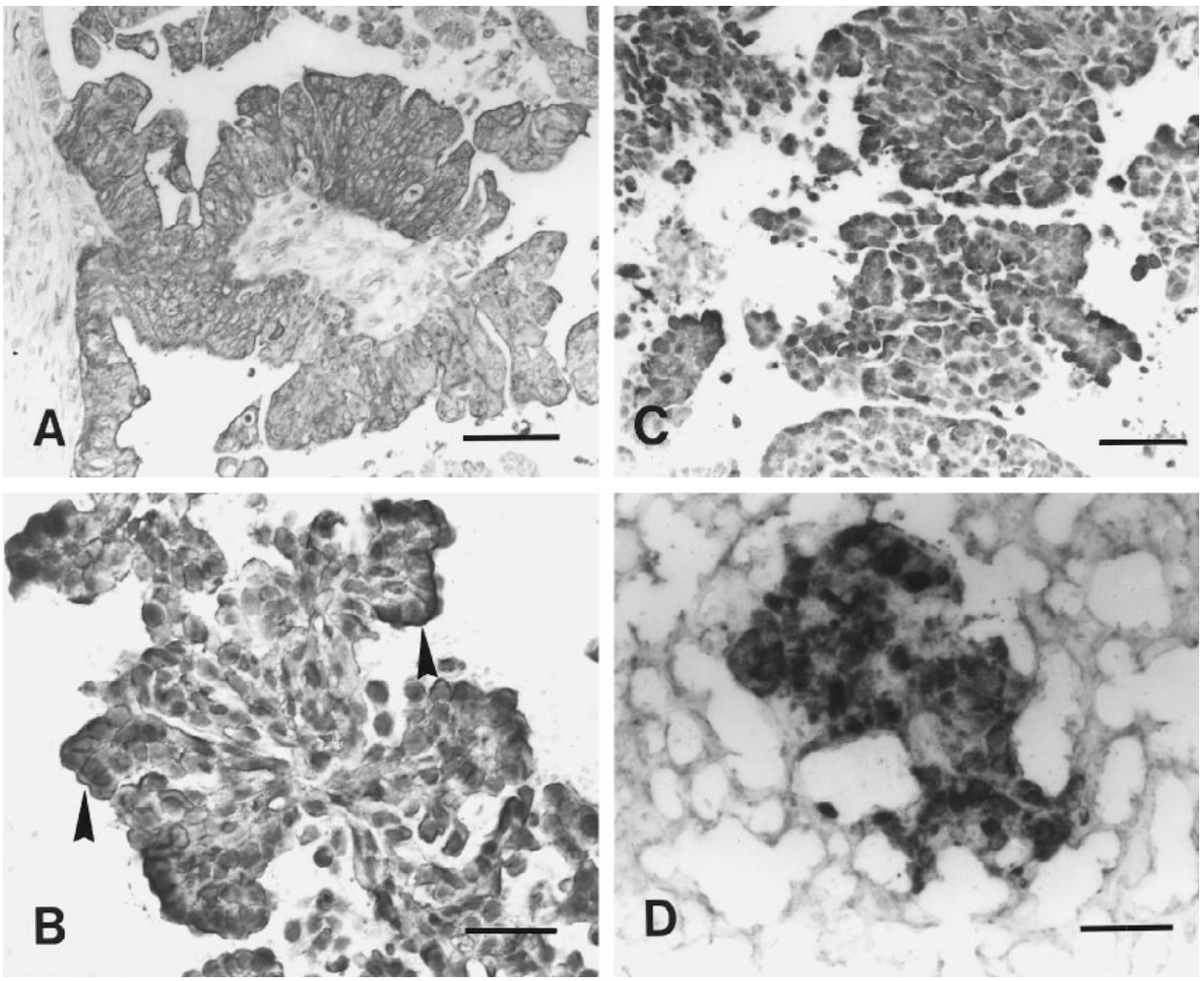

Figure 4. The lectin HPA binds to the patient tumour (A, bar 50 μm), to the SoTü cells grown intraperitoneally in suspensions, note the particularly strong reaction at the cellular apex (B, arrowheads; bar 50 μm), to the cells grown subcutaneously as a solid tumour (C, bar 50 μm) and to SoTü metastatic cells in the lung (D, bar 50 μm) indicating a stable expression of *N*-acetylgalactosamine residues under different growth conditions. Note the heterogeneity of HPA staining, however.

for the accumulation of glycogen were offered by her: (1) a higher amount of glucose in the medium as compared to the *in vivo* situation caused glycogen accumulation or (2) glycogen accumulation was viewed as a sign of dedifferentiation. Due to the breakdown of the blood – peritoneal cavity barrier in the ascites bearing mice, one would assume that the glucose concentration in the ascites is equal to or less than that in the blood. Hence an increased glucose level is an unlikely explanation for an increased glycogen accumulation within the SoTü cells. Since they grow as a differentiated solid tumour with cyst formation at the implantation site, the interpretation could be that glycogen accumulation in the SoTü cells growing in the ascites is a sign of dedifferentiation.

It is remarkable that the expression of most markers, including those for cell proliferation and expression of cell adhesion molecules, some of them thought to be involved in the metastatic cascade, were found to be similar in both the patient and the SCID mouse system, indicating that the environment for the growth of human ovarian cancer cells is ideal in SCID mice. A similar observation on a parallel growth behaviour study of human cells in patients and in SCID mice has been made using colorectal villous adenomas. Human colorectal villous adenoma engrafted for a prolonged period into SCID mice did not show any malignant transformation and hence behaved as it would in a patient (Bumpers *et al.* 1994). It is of particular interest that cells grown in suspension were able spontaneously to form solid tumours again when engrafted in the ovary or when

injected subcutaneously when the SoTü cells formed cysts as in the primary tumour. The cysts were, however, smaller in size than in the primary tumour or in tumours grown in the SCID ovary, a finding which emphasizes the role of the microenvironment for tumour growth and development, and which is in accord with the recent reappraisal of the tumour microenvironment (Fodstad & Kjonniksen 1994).

Lectin analysis of the primary tumour revealed intense reactivity of most if not all tumour cells with those lectins which were specific for a variety of sugars which are indicative of both *O*- and *N*-linked carbohydrate residues (Spicer & Schulte 1992). HPA binding sites were detected in the primary tumour; the intensity and the percentage of cells which were HPA positive increased in the ascites as compared to the original tumour. The presence of HPA binding sites in the original tumour as well as in the tumour cells grown in the SCID mice is of interest since HPA binding sites are associated with metastasis in clinical studies of human tumours including breast, stomach, prostate and colon (for references, see Schumacher *et al.* 1995a). HPA bound to the original tumour and since the patient later developed metastases it may be that HPA could be used as a prognostic indicator in ovarian cancer as well. The formation of distant metastases is a relatively unusual event in ovarian cancer (Dold & Sack 1980); however, it is of interest to note the similarity of the metastatic pattern in the patient and in SCID mice. This argument is strengthened by the observation that HPA has also been used as a marker of metastasis in a human colon cancer cell line transplanted into SCID mice, indicating that metastasis in SCID mice reflects the clinical situation (Schumacher *et al.* 1994). Comparing the marker pattern between the original patient tumour and the tumour grown in SCID mice is of particular importance when studying the metastatic phenotype, since different phenotypically stable clonotypes which were ultrastructurally indistinguishable from each other have been grown from one biopsy of a human non-small cell carcinoma of the bronchus and showed different levels of engraftment and metastases in SCID mice (Chen *et al.* 1994). The occurrence and stability of HPA binding sites as a possible marker of metastasis in ovarian cancer therefore deserves further investigation.

As the behaviour of the tumour cells in patients and in the SCID mice was very similar, this new model system is ideally suited to investigate the role of cell adhesion molecules in the metastatic spread of ovarian cancer. In our model system SoTü cells metastas-ized into the lungs, although only relatively few micrometastases were present. The somewhat limited ability to metastasize is correlated with strong E-cadherin immunoreactivity of the tumour cells and since e-cadherin is considered to be a tumour suppressor molecule which acts at the invasion site (Birchmeier & Behrends, 1994, Birchmeier, 1995), our findings would correlate well with previous observations. That the cells have metastasised despite the strong E-cadherin immunoreactivity might be due to the fact that E-cadherin expression, at least in mouse ovarian carcinoma cells, is unstable (Hashimoto *et al.* 1989) and hence some non-E-cadherin expressing cells have taken the opportunity to spread to the lungs. The use of E-cadherin knockout mutants in this clinically relevant SCID mouse model system would aid further investigation into the role of E-cadherin expression in the metastasis of ovarian carcinomas.

References

Astoul P., Colt, H.G., Wang, X. & Hoffman R.M. (1993) Metastatic human pleural ovarian cancer model constructed by orthotopic implantation of fresh histologically-intact patient carcinoma in nude mice. *Anticancer Res.* **13**, 1999–2002.

Birchmeier W. (1995) E-cadherin as a tumor (invasion) suppressor gene. *BioEssays* **17,** 97-99.

Birchmeier W. & Behrens, J. (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. *Biochim. Biophys. Acta* **1198,** 11-26.

Bumpers H.L., Alosco T.R., Wang H.-Q., Petrelli N.J., Hoover E.L. & Bankert, R.B. (1994) Human villous adenomas engrafted into scid mice survive for prolonged periods without malignant transformation. *J. Clin. Invest.* **94,** 2153-2157.

Chen F.A., Alosco T., Croy B.A., Narumi K., Percy D.H. & Bankert, R.B. (1994) Clones of tumor cells derived from a single primary human lung tumor reveal different patterns of beta 1 integrin expression. *Cell Adhes. Commun.* **2,** 345-357.

Dold U. & Sack H. (1980) *Praktische Tumortherapie*. Second edition, Stuttgart, Thieme, p 339.

Fenoglio C.M. (1980) Overview article: Ultrastructural features of the common epithelial tumours of the ovary. *Ultrastruct. Pathol.* **1,** 419-444.

Fodstad O. & Kjonniksen, I. (1994) Microenvironment revisited: time for reappraisal of some prevailing concepts of cancer metastasis. *J. Cell. Biochem.* **56,** 23-28.

Frei E. (1982) The national chemotherapy programme. *Science* **217,** 600-606.

Fu X. & Hoffman R.M. (1993) Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. *Anticancer Res.*, 283-286.

Garofalo A., Chirivi R.G., Scanziani E., Mayor J.G., Vecchi A. & Giavazzi R. (1993) Comparative study on the metastatic behaviour of human tumours in nude, beige/nude/xid and severe combined immunodeficient mice. *Invasion Metastasis* **13,** 82-91.

Hashimoto M., Niwa O., Nitta Y., Takeichi M. & Yokoro K. (1989) Unstable expression of E-cadherin adhesion molecules in metastatic ovarian tumor cells. *Jpn. J. Cancer Res.* **80,** 459-463.

HILLS C.A., KELLAND L.R., ABEL G., SIRACKY J., WILSON A.P. & HARRAP K.R. (1989) Biological properties of ten human ovarian carcinoma cell lines: calibration in vitro against four platinum complexes. *Br.J. Cancer* **59,** 527-534.

HSU M., RAINE L. & FANGER H. (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J. Histochem Cytochem.* **29,** 577-580.

MALIK S.T., MARTIN D., HART I. & BALKWILL F. (1991) Therapy of human ovarian cancer xenografts with intraperitoneal liposome encapsulated muramyl-tripeptide phosphoethanolamine (MTP-PE) and recombinant GM-CSF. *Br.J. Cancer* **63,** 399-403.

SCHUMACHER U., ADAM E., BROOKS S.A. & LEATHEM A.J. (1995a) Lectin-binding properties of human breast cancer cell lines and human milk with particular reference to Helix pomatia agglutinin. *J. Histochem. Cytochem.* **43,** 275-281.

SCHUMACHER U., ADAM E., FLAVELL D.A., BOEHM D., BROOKS S.A. & LEATHEM A.J. (1994) Glycosylation pattern of the human colon cancer cell line HT29 detected by Helix pomatia agglutinin and other lectins in culture in primary tumours and in metastases in SCID-mice. *Clin. Exp. Metast.* **12,** 398-404.

SCHUMACHER U., STAMOULI A., ADAM E., PEDDIE M. & PFÜLLER U. (1995b) Biochemical, histochemical and cell biological investigations on the actions of mistletoe lectins I, II and III with human breast cancer cell lines. *Glycoconjugate J.* **12,** 250-257.

SPICER S.S. & SCHULTE B.A. (1992) Diversity of cell glycoconjugates shown histochemically: A perspective. *J. Histochem. Cytochem.* **40,** 1-38.

VENDITTI J.M. (1983) The National Cancer Institute antitumour drug discovery programme. *Cancer Treat. Rep.* **67,** 767-772.

WALTER I. (1995) Culture of bovine oviduct epithelial cells (BOEC). *Anat. Rec.* **243,** 347-356.

WILLIAMS S.S., ALOSCO T.R., CROY B.A. & BANKERT R. (1993) The study of human neoplastic disease in severe combined immunodeficient mice. *Lab. Animal. Sci.* **43,** 139-146.

WILSON A.P. (1984) Characterization of a cell line derived from the ascites of a patient with papillary serous cystadenocarcinoma of the ovary. *J. Natl. Canc. Inst.* **72,** 513-521.