Virchows Archiv B Cell Pathol (1993) 63:335–343

Virchows Archiv B
Cell Pathology
Including Molecular Pathology
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Characterization of four new gastric cancer cell lines

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Received April 3, 1992 / Accepted February 20, 1993

Summary. Four well differentiated gastric adenocarcinoma cell lines from German patients have been established from primary tumors (St 23132, St 3051) and lymph node metastases (St 2474, St 2957). The tumor cells were isolated by enzymatic or mechanical treatment. All four lines grew as solid tumors in nude mice and formed colonies in soft agar. The doubling time of the cells in culture was 25–32 h. Further characteristics of the lines were a considerable chromosomal aneuploidy, (the chromosomal numbers varying from 30–109 with many numerical and structural abnormalities), a stable keratin expression (Ck 8, 18, 19), the expression and secretion of CEA and CA-19-9 and the overexpression of c-myc. The four stomach cancer cell lines described here are not only a useful addition to the small number of existing lines, but also represent ideal tools for studying tumorigenicity of human stomach cancers in vitro and in vivo.

Key words: Gastric carcinoma cell lines – Tumorigenicity – Keratin expression – c-myc

Introduction

Stomach cancer is still frequent worldwide (Correa 1985) and high rates are reported from Japan, China, CIS and parts of Latin America. In European countries the rates are intermediate to high (Mettlin 1988; MacDonald et al. 1985). Over 90% of malignant gastric tumors are adenocarcinomas and although more have been surgically resectable over the past few decades, the survival rate has not improved significantly. Furthermore, apart from the so far unexplained relationship between socioeconomic status and gastric cancer, no specific causative factors have been demonstrated. The search for therapeutic and diagnostic reagents and the investigation of cellular mechanisms involved in stomach cancer growth would be much facilitated if enough suitable cell lines could be developed. So far, only a few laboratories have successfully isolated and established gastric carcinoma cell lines (Hojo 1977; Motoyama et al. 1986; Laboisse et al. 1982; Lin et al. 1984; Akiyama et al. 1988; Park et al. 1990; Yoshida et al. 1989; Terano et al. 1991; Yanagihara et al. 1991; Dippold et al. 1991). The reasons why most stomach cancer cells do not grow in cell culture have not been fully explained, but one probable reason is the low proliferation rate of these cells (Yoshikawa et al. 1986; Vollmers et al. 1989). Many cultures simply become overgrown by contaminating fibroblasts after several passages. Furthermore, most gastric cells either do not respond or react only in a limited manner to external growth factors (Weinstock and Baldwin 1988).

In this paper we describe the establishment and characterization of four new gastric cancer cell lines from German patients with adenocarcinomas. These lines were established during a study of the humoral immune response in patients with gastric carcinoma (Vollmers et al. 1989; Pfaff et al. 1990; Faller et al. 1990). Of 125 preparations, about 50% were established as primary cultures, but only four as pure long-term cultures. The cell lines have been investigated morphologically, immunohistochemically and biochemically, and have been tested for tumorigenicity in soft agar as well as primary and metastatic growths in nu-nu mice. In addition, all four lines were examined for amplification and overexpression of cellular oncogenes, growth factor receptors and the production of tumor markers.

Material and methods

Cell Culture. Cell lines were established as previously described (Vollmers et al. 1989; Pfaff et al. 1990). Briefly, lymph node metastases were disrupted mechanically and the resulting suspension was cultured in RPMI-1640 with 10% FCS (fetal calf serum) at 37° C

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Abbreviations. FCS, fetal calf serum; *PBS*, posphate-buffered saline; *SDS*, sodium dodecyl sulfate

and 10% CO_2. Solid tumors were cut into 1–2 mm fragments and treated with hyaluronidase type II (Sigma, Munich, Germany) (580 units/ml) and collagenase type I (480 units/ml) for 20 min at 37° C. The digested tumor fragments were filtered through a 100 µm mesh, washed with RPMI-1640 and cultured in RPMI-1640 containing 10% FCS, 1% penicillin/streptomycin.

Immunoperoxidase staining on cells. Cytospin preparations (5000 cells/slide) were fixed with acetone, washed three times with Tris buffer (pH 7.4), incubated with the diagnostic antibodies (Dianova, Hamburg, Germany, except anti-Ck 7, 8, 13, 18, 19, Boehringer, Mannheim, Germany) and diluted in Tris containing 0.5% BSA, for 30 min at room temperature. The slides were washed twice with Tris, incubated with peroxidase-coupled rabbit antibodies to mouse-Ig (Dakopatts, Hamburg, Germany), and diluted 1:50 in PBS containing 30% AB-Rh-positive human serum for 30 min at room temperature. The slides were washed again twice with Tris (pH 7.4), once with Tris (pH 7.6), and then incubated with substrate (0.006% diaminobenzidine and 0.015% hydrogen peroxide) for 10 min. The cells were briefly counterstained with hematoxylin. CEA and CA 19-9 levels in the supernatants of the cells were determined using a CEA-kit from Abbott, Wiesbaden, Germany and a CA 19-9-kit from CIS bioindustries, Paris, France.

Karyotype analysis. Growing cells were incubated for 60 min with 10 µl/ml colcemid (Gibco, Eggenstein, Germany) at 37° C. The cells were then trypsinized, pelleted and incubated with 15 ml of warm 0.8% Na Citrate/0.075 M KCL solution for 15 min at 37° C. The cells were then centrifuged, fixed with methanol/acetic acid (4:1) and stored at − 20° C. For G-banding experiments, pelleted cells were dropped on cold slides and dried for 1 week at room temperature. The slides were then incubated in PBS (50 ml, containing 175 µl Bacto-Trypsin) for 75 sec, washed with PBS for 75 sec and stained with 7% Giemsa solution for 8 min. For R-banding experiments the slides were washed in distilled water and incubated in 1.25 M NaH2PO4 solution (87.2° C) for 15 min. After short washing steps, the slides were stained for 15 min in 7% Giemsa-solution (Saal et al. 1988).

In vitro and invivo growth experiments. For soft agar experiments, tumor cells were first trypsinized and washed. Cells (n = 2–3000) were then plated on 3 cm Petri dishes containing 0.6% soft agar (Vollmers et al. 1985) and grown at 37° C and 10% CO_2. For growth kinetics 10 trypsinized tumor cells were plated on 24- well culture plates, grown for 1 week and counted every day. For in vivo experiments on solid tumor growth, 2×10^7 tumor cells were injected subcutaneously into NMRI nu-nu mice (Zentralinstitut für Versuchstierforschung, Hannover, Germany). After 4–6 weeks, the tumors were excised and either frozen in liquid nitrogen, fixed in formalin or used for cell culture. To investigate metastatic growth, 10^6 the organs were inspected macroscopically and histochemically for metastatic lesions (Vollmers and Birchmeier 1983).

Enrichment of intermediate filament proteins. Cytoskeletal proteins were prepared as described by Gown and Vogel (1982) in the presence of 1 mM PMSF. This preparation represents the cellular material insoluble in solutions containing 1% Triton-X-100 and with high ionic strength (1.5 M KCl). Cytokeratin subtypes were identified after one- and two-dimensional SDS-polyacrylamide gel electrophoresis by the criteria of molecular weight, isoelectric point and reactivity with commercially available cytokeratin subtype specific antibodies in Western blots (as outlined by Moll et al. (1982).

Electrophoretic procedures. SDS-polyacrylamide (10%) gel electrophoresis was performed as described by Laemmli (1970). Two-dimensional electrophoretic separations were carried out as isoelectric focusing in the first dimension and SDS-polyacrylamide gel electrophoresis in the second dimension (O'Farrell 1975). SDS-denatured samples were applied for isoelectric focusing as described by Comings and Peters (1981).

Western blotting. Electrophoretic transfer of separated components from polyacrylamide gels to 0.45 µm nitrocellulose membranes (Schleicher and Schüll, Dassel, Germany) was performed in a semi-dry blotting apparatus (LKB, Freiburg, Germany) according to the manufacturers directions. Thereafter, the dried membrane was treated with PBS containing 10% FCS and 0.5% Tween 20 (buffer A) for 1 h at room temperature followed by a 2-h incubation with the first antibody diluted in blocking buffer to a concentration of 1–10 µg/ml. After five washes with PBS 0.05% TWEEN 20 (washing buffer), peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dako) were added in buffer A at a dilution of 1:1000. After a 2 h incubation, the nitrocellulose membrane was washed four times with washing buffer and once with TBS. Color was developed in a freshly prepared mixture of 20 ml TBS, 4 ml 4-chloro-1-naphthol (3 mg/ml in methanol) and 5 µl hydrogen peroxide. Protein staining on nitrocellulose membranes was performed with a solution of 0.1% Pelikan india ink type 17 (Pelikan, Hannover, Germany) and 0.5% TWEEN 20 in PBS, with subsequent washing in washing buffer (Hancock and Tsang 1983).

RNA preparation and Northern blotting. Frozen tissue was homogenized and suspended in guanidine thiocyanate buffer, followed by caesium-chloride gradient centrifugation. Total RNA (20 ng per lane) was size fractionized by electrophoresis in 6% formaldehyde/ 1.5% agarose gels. Before RNA transfer to nylon membranes (Hybond, Amersham, Germany), the gels were stained with ethidium bromide to verify equal quantities of RNA. Specific probes of c-myc, c-myb, H-ras, c-sis, EGF-R and erb-B were obtained from Dianova, Germany. The probes were tail-labeled with alpha 32-P-dATP using terminal transferase (TdT, Gibco, Eggenstein, Germany). Briefly, 4 µl 5 × tailing buffer (shipped with the enzyme), 50–100 µCI dATP, 25 U of TdT and 16 µl double-distilled water were mixed and incubated at 37° C for 2 h. The unincorporated nucleotides were removed by NH4-acetate-ethanol precipitation. The specific activity of the oligonucleotides was $5 \times 10^7 - 2 \times 10^8$ cpm/µg. The nonradioactive probes were tested on nitrocellulose spotblots. Probes detectable at a minimum of 1–10 pg were used in the hybridization experiments. Prehybridization was performed in 50% formamide, 2 × SSC, 0.1% SDS, 20 mM vanadyl-ribonucleoside-complex, 200 µg/ml salmon sperm DNA (Boehringer, Mannheim, Germany), 200 µg/ml yeast-tRNA (Boehringer, Mannheim, Germany), 5 × Denhardts solution, 10% dextran sulfate for 4–6 h at 58° C. Alpha 32-P-labeled anti-sense-oligonucleotide probe (1–2 ng/ml) was added and hybridization was performed for 12–16 h. Filters were washed twice for 5 min with 2 × SSC, 0.1% SDS at room temperature, twice for 30 min with 2 × SSC, 0.1% SDS at 65° C and twice for 10 min at 65–70° C. Filters were then exposed to Kodak-X-omat films (Kodak, New York, USA) for 8–72 h (Sambrook et al. 1989).

DNA preparation and Southern blotting

Probes were labeled with 32-P alpha-dATP (NEN, DuPont, France) by terminal desoxynucleotidyl transferase (Gibco, BRL, Germany) 1 h at 37° C. Hybridization was performed in 50% formamid, 10% dextran-SO4, 5 × Denhardts, 4 × SSC, 10 mM TRIS, 10 mM EDTA, 0.1% SDS and 100 µg/ml herring sperm DNA at 42° C overnight. Specific activity of the solution was adjusted to $2\text{–}10^8$ cpm/ml. The membranes were washed for 30 min at 60° C in 2 × SSC and 2 times for 15 min in 0,2% SSC, 0.1% SDS. Films were exposed for 4–24 h (Sambrook et al. 1989).

Ultrastructural studies. For ultrastructural studies cells were grown to confluence on Petri dishes, washed with PBS and fixed with 3% glutaraldehyde. Sections from the monolayer were cut out and processed for electron microscopy.

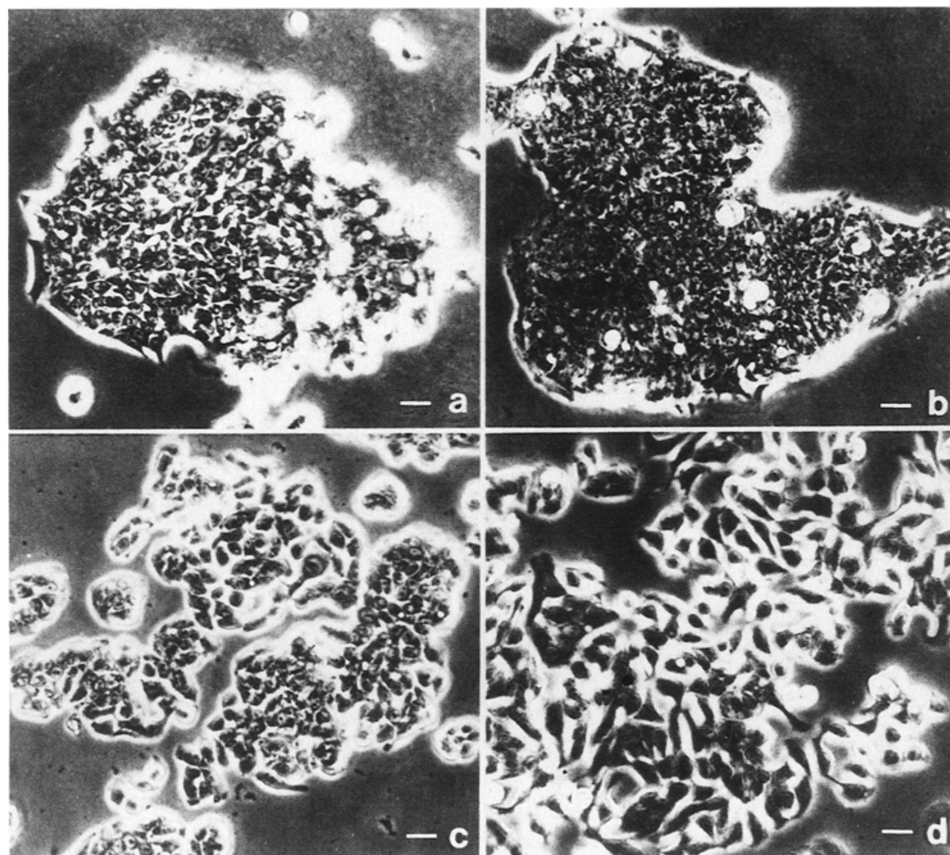

Fig. 1a–d. Morphology of stomach carcinoma cell lines. **a** St 23321; **b** St 2474; **c** St 2957; **d** St 3051. *Scale bar* = 200 μm

Table 1. Patients

Cell line	Date of culture	Patient age/sex	Prior therapy	Source[a] of cells	Tumor		
					Grade	Stage	Type Lauren
St 23132	11/87	72/M	None	primary	G3	pT2,pN0	intest.
St 2474	2/90	60/M	None	metast.	G2	pT1,pN2	intest.
St 2957	2/90	51/M	None	metast.	G3	pT2,pN2	intest.
St 3051	2/90	66/M	None	primary	G3	pT4,pN2	intest.

[a] Tissue used for cell culture; primary: stomach tumor; metast.: lymph node metastases

Results

Growth characteristics

From more than 100 freshly isolated gastric carcinoma specimens from German patients, short-term primary cultures were obtained from 50%, but most of the cells stopped growing after several passages or were overgrown by contaminating fibroblasts. However, four long-term cell lines were established from primary tumors (St 23132, St 3051) and lymph node metastases (St 2474, St 2953) from male patients (Table 1). All four lines are mycoplasma-free and one (St 23132) has now been growing for more than 2 years in culture. The epithelial cells show adherent growth (Fig. 1 and Table 2) in RPMI 1640 with 10% FCS without any additional supplements; they have doubling rates of between 18 and 31 h and reach cellular densities of between 0.8 and 2×10^6 cells/ml (Table 2). No changes in morphology or growth behavior have been observed. Ultrastructural studies (Fig. 2) show that the cell line St 23132 expresses irregularly arranged microvilli on the cell surface, in the intercellular space and in intercellular junctional complexes. This latter characteristic is also seen in the other three cell lines (Fig. 2a). St 2474 cells show enlarged nuclei with prominent nucleoli and many focally accentuated glycogen granules (Fig. 2b); St 2953 also expresses microvilli on the surface and lysosomal granules are present in the cytoplasm (Fig. 2c). A feature typical of cell line St 3051 is the presence of numerous mucinous granules and intracellular acinar lumina surrounded by microvilli (Fig. 2d). When tested for growth in semisolid

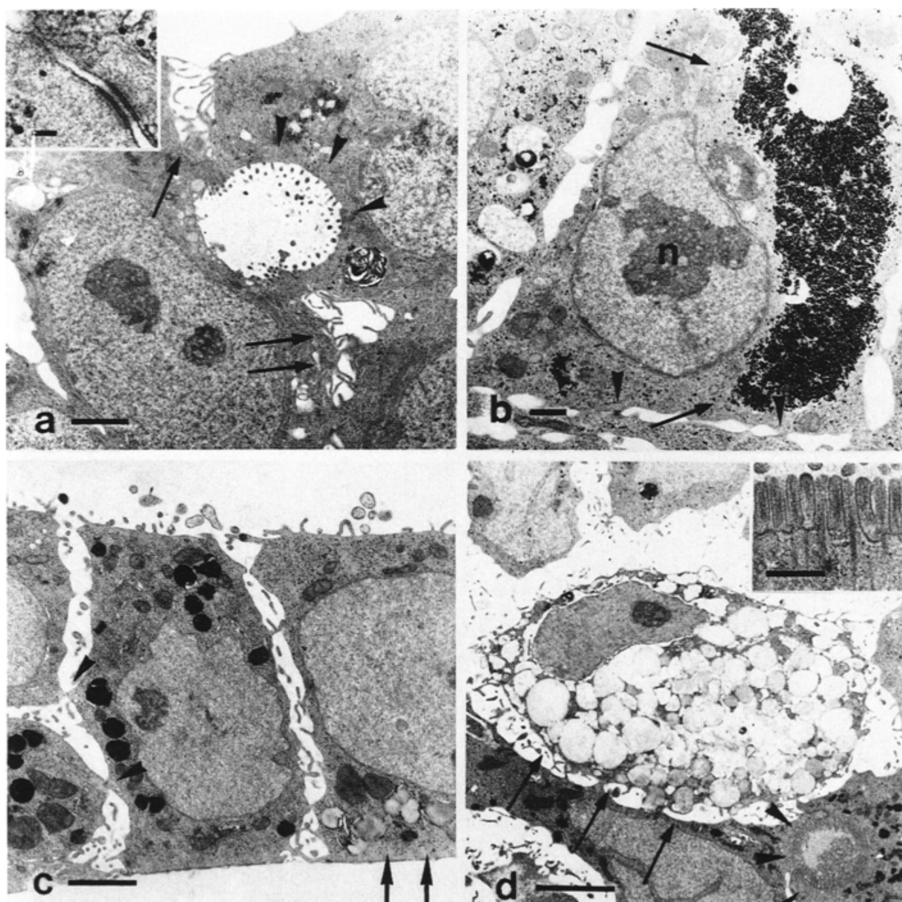

Fig. 2a–d. Ultrastructural morphology of stomach cancer cell lines. **a)** St 23132 (×4800): microvilli at the surface (*one arrow*), and in the intercellular space (*two arrows*), intercellular lumen (*arrow heads*), intercellular junctional complexes (*inset*, ×48000). *Scale bar* = 2 μm **b)** St 2474 (×7000): note enlarged nuclei with prominent nucleoli (*n*) and many focally located glycogen granules (*arrows*); *Scale bar* = 2 μm **c)** St 2957 (×6000): note microvilli at the surface and in the intercellular space as well as junctional complexes (*arrowheads*); in addition organelles such as secondary lysosomes (*arrows*) and granules containing mucin or zymogen; *Scale bar* = 2 μm **d)** St 3051 (×3000): some tumor cells contain numerous mucinous granules (*arrows*). The nucleus is displaced near the cellular membrane; an intracellular lumen can be seen (*arrowheads*) surrounded by microvilli, which is reproduced as inset (×19000). *Scale bar* = 2 μm. *Scale bar* in *insets* = 0.2 μm

Table 2. Cellular growth characteristics

Cell line	Growth type	Doubling time/h	Cell density	Tumorigenicity		
				Soft agar	nu-nu tumor	nu-nu metastases
St 23132	Adherent	30–32	2×10^6/ml	+ [a]	+ [b]	– [c]
St 2474	Adherent	20–24	8×10^5/ml	++	+	–
St 2957	Adherent	20–24	1×10^6/ml	++	+	–
St 3051	Adherent	18–20	1×10^6/ml	+	+	–

[a] +: Visible colonies after 10–14 days; ++: after 5–7 days;
[b] +: Solid tumors (1 cm) after 3–4 weeks,
[c] Metastases formation in nu-nu mice was examined morphologically 4–6 weeks after subcutaneous injection of tumor cells

medium, St 2474 and St 2957 show a high plating efficiency (nearly 40–60%) and form visible colonies after 1 week, whereas with St 23132 and St 3051, only about 10% of the plated cells grow as colonies after a period of 2 weeks. No marked differences were seen in the nude mice experiments. Following subcutaneous injection, all cell lines form solid tumors (1 cm) after 3–4 weeks. Between five and ten mice were tested for each cell line and the efficiency of growth was 90–100%. No metastases were observed with any of the four cell lines.

Immunohistochemical characterization

To study the phenotype of the four gastric cell lines and possible changes during growth in agar and nude mice, cytospin preparations were stained with various antibodies against differentiation markers. All cell lines were found to be positive for HLA-ABC, Ki-67 and keratins 8, 18 and 19. In addition, the cells secrete CEA and Ca 19-9, as determined in the supernatants (Table 3). No significant differences were observed between the primary cultures, the soft agar colonies and the nude mice tumor cells. This indicates a relative stability of the gas-

Table 3. Immunohistochemical characterization of cell lines

Marker	Cell line											
	St 23132			St 2474			St 2957			St 3051		
	pr.	nu.	ag.	pr.	nu.	ag.	pr.	nu.	ag.	pr.	nu.	ag.-
HLA-ABC	+	+	+	+	+	+	+	+	+	+	+	+
HLA-DR	–	–	–	–	–	–	–	–	–	–	–	–
Leu 1	–	–	–	–	–	–	–	–	–	–	–	–
EMA	–	–	–	–	–	–	–	–	–	–	–	–
CEA	+	+	+	+	+	+	+	+	+	+	+	+
CA 19-9	+	+	+	+	+	+	+	+	+	+	+	+
Vimentin	–	–	–	–	–	–	–	–	–	–	–	–
Ck 8	+	+	+	+	+	+	+	+	+	+	+	+
Ck 18	+	+	+	+	+	+	+	+	+	+	+	+
Ck 19	+	+	+	+	+	+	+	+	+	+	+	+
Ck 7	–	–	–	–	–	–	–	–	–	–	–	–
Ck 13	–	–	–	–	–	–	–	–	–	–	–	–

pr.: primary culture; *nu.*: nu-nu mouse tumor; *ag.*: agar colonies. CEA and CA 19-9 antigens were determined in cellular supernatants, all other markers on cytospin preparations or biochemically. (Levels of CEA/1 Mill. cells: 23132: 37.7 ng/ml/d; 2474: 1.1 ng/ml/d; 2957: 1.0 ng/ml/d; 3051: 0.4 ng/ml/d) (Levels of CA 19-9/1 Mill. cells: 23132: 6 U/ml/d; 2474: 2.5 U/ml/d; 2957: 8 U/ml/d; 3051: 4 U/ml/d)

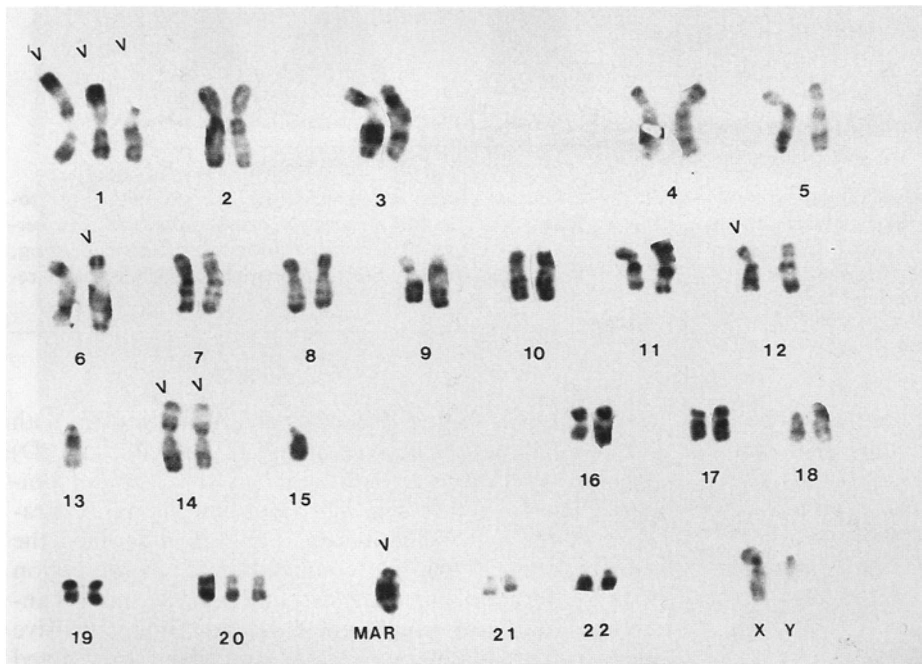

Fig. 3. Karyotype of St 23132. Chromosomal abnormalities are indicated by *arrows*

tric cancer cells growing under different conditions. No expression of class II antigens or vimentin was observed.

Karyotyping

All four cell lines derived from male patients had near diploid chromosome numbers ranging from 47–51 (Table 4). The cells differ in the percentage of the modal chromosome number, e.g. 40% of St 23132 have 47 chromosomes, whereas only 10% of St 2474 have 50 and from St 2957 and St 3051 only 4% show modal numbers of 51 and 48 chromosomes, respectively. A se-

Table 4. Cytogenetic studies

Cell line	Modal chromosome no.	Ranges	% of cells with modal numbers	% of cells with higher ploidies
St 23132	47	44–52	42	21
St 2474	50	31–109	10	32
St 2957	51	37–86	4	32
St 3051	48	34–86	4	50

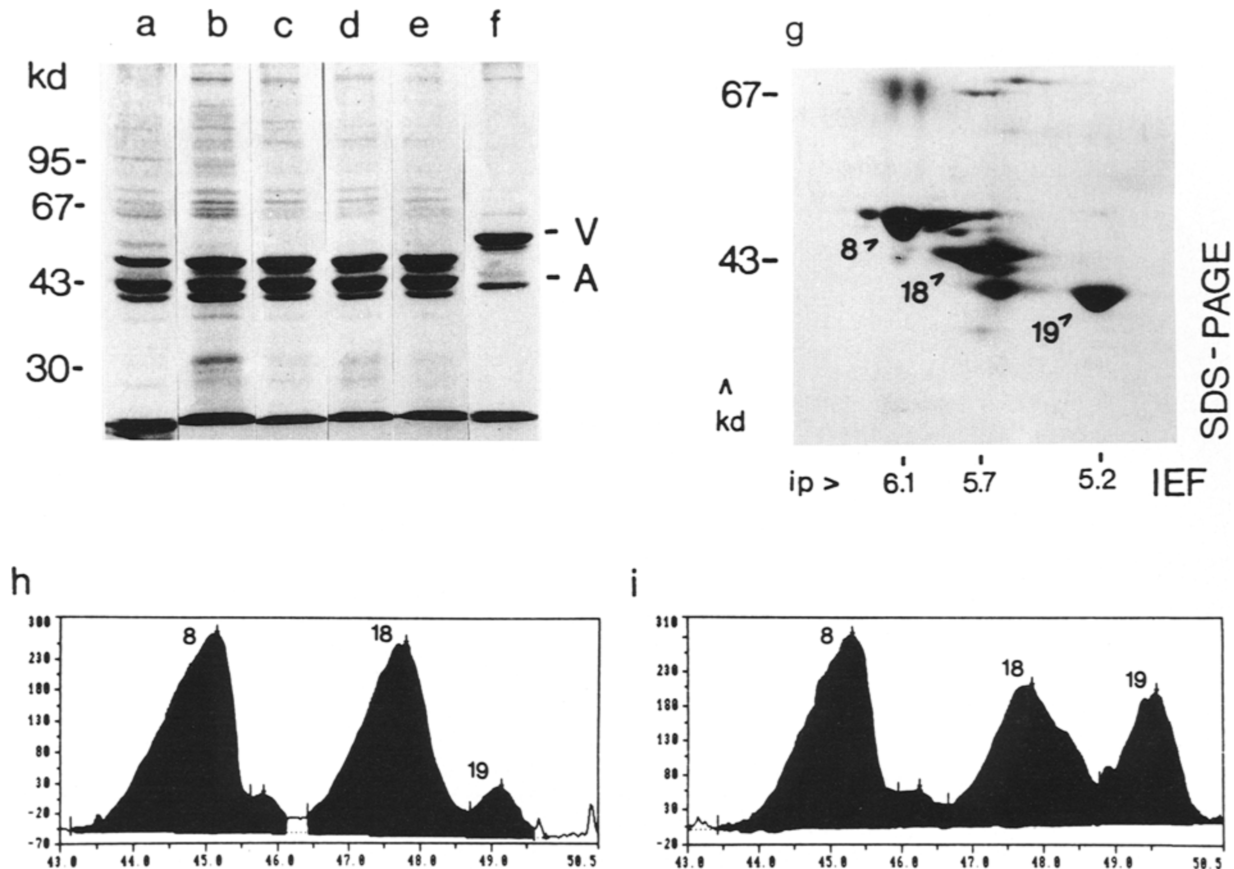

Fig. 4. Biochemical characterization of cytoskeletal preparations of cultured cells. (*a–f*) Coomassie-stained SDS-polyacrylamide electrophoresis; (*g*) two-dimensional separation of St 2474 cytoskeleton (Coomassie-stained gel); (*h, i*) densitometric scans of lanes (*c*) (St 2957) (*h*) and (*b*) (St 2474) (*i*). Cytoskeletal preparations are from St 23132 (*a*), St 2474 (*b, g, i*), St 2957 (*c, h*), St 3051 (*d*), HT29 (*e*) cells and foreskin fibroblasts (*f*). *kd*: kilodalton; *ip*: isoelectric point; *V*: vimentin; *A*: actin; 8: cytokeratin 8; 18: cytokeratin 18; 19: cytokeratin 19; IEF: separation by isoelectric focusing; SDS-PAGE: separation by SDS-polyacrylamide gel electrophoresis

ries of numerical and structural abnormalities were seen in the karyotypes of the adenocarcinomas. Significant aberrations were noted, such as 47X,Y; inv1 (q31,p22); t(1,15)(1ptel-cen, 15qcen-tel) (1qtel-cen,15p); (6qtel-cen, 12q14-tel) (6ptel-cen,12p-q14); t(13,14) t(14q, marker1); and +20;+marker2 (Fig. 3). Similar results were obtained with the three other stomach cancer lines (data not shown).

Biochemical characterization

The data on keratin expression after immunohistochemical staining (Table 3) were further confirmed by biochemical analysis of cytoskeletal preparations (Fig. 4). Cytoskeletons were prepared by extraction of the cells with buffers containing nonionic detergent (1% Triton X-100) and high salt concentrations (1.5 M KCl). The insoluble residues of these extraction procedures were subjected to SDS-polyacrylamide gel electrophoresis. The polypeptide composition of the cytoskeletons of all four stomach cancer lines (Figs. 4a–d) is very similar to the intermediate filament proteins present in the colon cancer line HT29 (Fig. 4e), which is reported to express cytokeratins 8, 18 and 19 exclusively. A polypeptide with a molecular weight corresponding to vimentin (58 kD) was not detected in any of these cancer cell lines (compare Figs. 4a–e with Fig. 4f). Two-dimensional separations of the cytoskeletons (e.g. Fig. 4g) underlined the similarity in polypeptide composition. In combination with Western blotting analysis using subtype specific antibodies (data not shown), the data allowed a conclusive identification of the cytokeratin subtypes (as indicated in Fig. 4g). Densitometric scans of the one-dimensionally separated cytoskeletons (Fig. 4a–e) revealed one particular difference between the stomach cancer cell lines in that 2474/90 cells express significantly more cytokeratin 19 than cytokeratin 18, compared with HT29, St 23132, St 2957 and St 3051 cells (Figs. 4h, i).

Gene expression

Protooncogenes and growth factor receptors are often overexpressed in malignant cells. All four cell lines express high levels of c-myc RNA and three lines of EGF-receptor RNA (Fig. 5a, b; Table 5) compared with control cells or normal stomach tissue from the same pa-

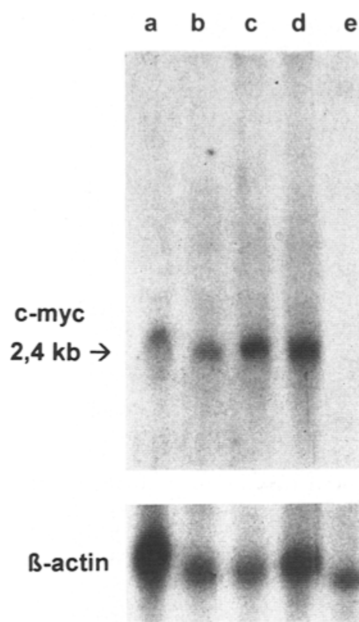

Fig. 5. Northern blots with RNA from stomach carcinoma cell lines and control cells and tissue: on the top) Hybridization with c-myc-probe: (*a*) St 23132. (*b*) St 2474. (*c*) St 2957. (*d* St 3051, *e*) normal stomach mucosa; bottom) Hybridization with β-actin probe, same order

Table 5. Northern blots

Probe	Cell lines			
	St 23321	St 2474	St 2957	St 3051
c-myc	+	++	++	++
c-myb	–	–	–	–
c-ras	–	–	–	–
erb-B-1	–	–	–	–
c-sis	–	–	–	–

tients. No expression of erb-B, c-myb, H-ras and c-sis RNA was seen. Amplifications or rearrangements of the above mentioned genes were not noted (data not shown).

Discussion

Successful investigation of the processes involved in tumor proliferation and metastasis formation ultimately depends on in vitro and in vivo studies with established tumor cell lines. We describe in this paper the characterization of four new stomach cancer cell lines, isolated from German patients. Briefly summarized, the cell lines show a high proliferation rate, grow in soft agar and nude mice, have a near diploid chromosomal set, express epithelial cell specific keratins, secrete CEA and CA-19-9 and most interestingly, show an overexpression of c-myc RNA. Only a few gastric carcinoma cell lines that grow as long-term cultures have been established in the last few years. Terano et al. (1991) described a new cell line derived from a signet ring cell carcinoma of the stomach that, like our cell lines, showed high secretion rates for CEA and CA-19-9. Another gastric adenocarcinoma cell line (TMK-1), isolated by Yoshida et al. (1989) showed a proliferative response to exogenous EGF and a seven-to-eight fold increase in EGF-R receptor mRNA after stimulation. Most difficult to establish are signet ring cell carcinomas of the stomach, because of their slow proliferation rate. However, Yanagihara et al. (1991) described two signet ring cell carcinomas that had an abnormal high doubling rate of about 28–30 h, and Dippold et al. (1991) increased the gastric carcinoma cell proliferation rate by using Il-3 and granulocyte-macrophage colony-stimulating factor. A major reason for the low success rate is that gastric carcinomas cannot be stimulated sufficiently by external factors. One exception, described by Weinstock and Baldwin (1988), is the stimulation of gastric carcinoma cells with gastric 17. However, there is evidence that the trophic effect of gastrin is mediated in this particular case by a high affinity receptor, whereas most cell lines arising from epithelial tumors express low affinity receptors for gastrin and show no proliferative response to it. There have been very few studies on the cytogenetics of gastric cancers, and so far no single specific chromosomal abnormality has been described. Cytogenetic investigation of our tumor cell lines revealed many aberrations similar to those in adenocarcinomas of other origins (Ferti-Passantanopoulou et al. 1987, 1988). However it can be said that St 2747, St 2957 and St 3051 have numerous marker chromosomes in common, e.g. isochromosome 13, der1, and trisomy 20 (Saal et al. 1988). These findings are epiphenomena rather than primary changes in the carcinoma cells, but they may influence tumor progression and malignancy.

Ultrastructural studies of the cell lines yielded one special finding, so far not reported for other gastric cancer cell lines. This was the formation of gland-like intercellular spaces observed in the cell line St 23132. The cell membrane of these cells shows a polar differentiation with a luminal part containing microvilli of the intestinal type and secretory vacuoles surrounding the glandlike structures. The lateral membrane is characterized by junctional complexes, including desmosomes and basal membrane by hemidesmosome-like structures adhering to the culture dishes, as demonstrated in vertical sections by Müller et al. (1988).

The cytokeratin subtypes present in the cytoskeletons of the four cell lines are typical of those observed in cells derived from simple and non-squamous epithelia. An expression of cytokeratins 8, 18 and 19 is routinely detected in adenocarcinomas of the intestinal tract and associated glands (Moll et al. 1982). Cytokeratin 8 always exists in pairs with a cytokeratin of the acidic subgroup, like cytokeratins 18 and 19 (Moll et al. 1982). The relative amounts of cytokeratin 8/18- and 8/19-filaments in the stomach cancer cell lines may reflect the differentiation state of the cell from which the tumor originated. Therefore, the high expression of cytokeratin 19 in St 2474 cells might indicate a distinct origin of the corresponding tumor distinct from that of other

stomach cancer cell lines as well as the colon carcinoma cell HT29.

Three of the cells described in this paper (St 2474, St 2957 and 3051) show an overexpression of c-myc RNA, whereas probes against H-ras, c-myb, c-sis and erb-B-2 gave no positive binding. Amplification and/or expression of cellular oncogenes in human carcinomas is described for c-myc in human lung cancer (Little et al. 1983), in stomach cancers (Nakasato et al. 1984; Shibuya et al. 1985), in colon carcinomas (Erisman et al. 1985), for erb-B-2 in gastric tumors and cell lines (Yamamoto et al. 1986) and for H-ras and Ki-ras in gastric adenocarcinomas (Ohuchi et al. 1987; O'Hara et al. 1986; Bos et al. 1986). Increased expression of c-myc seems to be a common phenomenon observed in malignant cells and may play a role in growth control and rapid proliferation (Shibuya et al. 1985; Erisman et al. 1985; for review see also Tahara 1990).

Taken together, the characteristics of the four new stomach cancer cell lines described here, including their morphological, genetical and biochemical features, show that these cell lines are unique compared with other published stomach cancer cell lines. They show a high proliferation rate, grow in soft agar and nu-nu- mice, express tumor markers and do not need external factors for proliferation, indicating an autocrine stimulation by growth factors or oncogene products. They may, therefore, be helpful in studying and understanding one of the most frequent carcinomas worldwide.

Acknowledgements. This work was supported by Dr. Mildred-Scheel-Stiftung/Deutsche Krebshilfe e.V.. We thank A. Halbman, I. Weiglein, M. Ionescu, C. Ringdörfer, E. Wocniak, E. Werder and H. Schmitt for excellent technical assistance.

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