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ORIGINAL ARTICLE

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Establishment and characterization of the follicular thyroid carcinoma cell line ML-1

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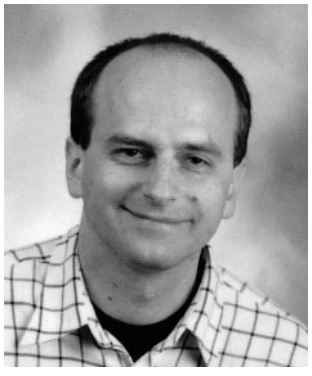

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Abstract The present study focuses on the establishment and characterization of a new follicular thyroid carcinoma cell line. The human cell line ML-1 was derived from a dedifferentiated follicular thyroid carcinoma relapse, which progressed despite preceding surgery followed by two radioiodine therapies. More than 90% of the cells of this line express thyroglobulin, chondroitin sulfate, and vimentin antigens, but only about 70% show cytokeratin filaments and a negative surface charge density such as human erythrocytes. More importantly, cells of this line are able to take up iodine and/or glucose both in vitro and in vivo and to secrete thyroglobulin, chondroitin sulfate, and fibronectin into the interstitial space. In addition, triiodothyronine is released constitutively into culture supernatants. Moreover, it is also suitable for xenotransplantation studies because it is tumorigenic in NMRI nude mice in vivo. The cell line forms tumors with follicular structures when transplanted to nude mice. Due to these unique features the ML-1 cell line can be considered as a very suitable test model for pharmacological and cell biological studies. Since chemicals may interfere with the production of thyroid hormones, this cell line represents also a tool for toxicological investigations.

Key words Human cell line · Follicular thyroid carcinoma · Iodine metabolism · Glucose metabolism · Triiodothyronine

Abbreviations *BISTRIS* 2,2-Bis (hydroxymethyl)-2,2',2''-nitrilotriethanol · *EPPS N*-(2-Hydroxyethyl) piperazine-*N*'-(3-propanesulfonic acid) · *FCS* Fetal calf serum · *FITC* Fluorescein isothiocyanate · *fT3* Free triiodothyronine · *fT4* Free thyroxine · *PBS* Phosphate-buffered saline · *TSH* Thyroid-stimulating hormone

Introduction

Follicular thyroid carcinomas are malignant epithelial tumors which express follicular patterns and normally are encapsulated [1]. They spread preferentially via blood vessels and form metastases in distant organs [2]. Usually, primary tumors are removed surgically as far as possible. Subsequently, residual tumors, metastases, and recurrent tumor are treated by radioiodine [3]. However, a considerable number of patients with progressed or dedifferentiated tumors do not respond to this type of therapy. The outcome in these patients greatly depends on the intensity of accumulation of the radioiodine in the thyroid carcinoma cells and has worse prognosis if tumor cells have lost their capability for radioiodine uptake due to dedifferentiation. Therefore several attempts have been made to induce redifferentiation of thyroid tumor cells in order to reactivate their iodine uptake capability [4, 5, 6, 7]. In this regard retinoic acid has shown quite promising effects when applied in vitro as well as in vivo. Thus a better understanding of the iodine uptake characteristics of thyroid tumor cells, especially of its negative correlation with glucose metabolism [8], may help in judging the value of therapeutic protocols with more accuracy and may facilitate the finding of ways to apply this kind of therapy as early as possible, which is most useful for the patient [9, 10].

Suitable cell lines consisting of cells with active iodide transporters [11] and/or with a high glucose-uptake capability [8] would provide an in vitro system for further studies on intracellular iodine accumulation by human follicular thyroid carcinomas. Although human follicular thyroid carcinoma cell lines have already been established [12, 13], intracellular iodine accumulation by cells of follicular thyroid carcinomas have thus far been studied using the rat thyroid cell line FRTL-5 [14] or short-term cultures derived from human follicular thyroid carcinomas [15]. The cell line ML-1 described in this paper may be useful for improving thyroid cancer therapy since the cells derived from a locally recurrent tumor of a poorly differentiated human follicular thyroid carcinoma can be stimulated by the thyroid-stimulating hormone (TSH) to incorporate ^{131}I for a period of 24 h. Release of both their thyroglobulin and their triiodothyronine (fT3) into the supernatant is, even after 25 passages, so efficient that reliable monitoring is possible.

Materials and methods

Case report

A 50-year-old woman presented in November 1996 a follicular thyroid carcinoma (stage T4) that was locally invasive into the fat and muscle tissue and had already spread into the lung. At that time she had a nearly total tumorectomy and 4 weeks later the first radioiodine therapy (3.16 GBq ^{131}I). A second therapy followed in May 1997 using 6.32 GBq ^{131}I. In September 1997 she returned to our hospital with a locally recurrent tumor and lymph node metastases. In a further operation a great part of the recurrent tumor was resected, and a specimen of it was used to establish the new cell line. After this surgery a third radioiodine therapy followed (5 GBq ^{131}I), but the patient died 4 months later.

Isolation and establishment of the cell line

The follicular thyroid cell line ML-1 was cultured according to a protocol successfully applied for establishing the papillary thyroid ONCO-DG-1 cell line [16]. In brief, a piece of malignant tumor tissue (1 cm^3) was obtained from a locally recurrent tumor of a poorly differentiated follicular thyroid carcinoma (pT4N1M1, grade 3) of the patient described above. The specimen was derived from the center of the recurrent tumor and mechanically dissociated and then treated with 2 ml of a mixture of 0.1% collagenase and dispase (Roche, Mannheim, Germany) for 30 min at 37°C in 5% CO_2 to obtain a single cell suspension. Afterwards cells were spun for 10 min at 2000 rpm, and the pellet was resuspended in tissue culture medium. Some of the cells were plated in culture flasks (Greiner, Frickenhausen, Germany), and the rest were stored in liquid nitrogen for future use (P_0). The cells were grown as monolayer cultures at 37°C in a humidified atmosphere of 5% CO_2. The medium was changed at intervals of 3 days. Cell viability was tested with the trypan blue method. Mycoplasma testing of cultures and controls was negative.

Tissue culture media

The medium used was Dulbecco's modified Eagle's medium (PanSystems, Nuremberg, Germany) containing 100 µM sodium pyruvate, 2 mM L-glutamine, 1 mg/ml glucose, and 3.7 g/l $NaHCO_3$. It was supplemented with 10% fetal calf serum (FCS; Gibco, Eggenstein, Germany), 100 U penicillin/ml, and 100 µg streptomycin/ml (PanSystems).

Multicellular tumor spheroids

To test a possible spheroid formation capability of the ML-1 cells the procedure was performed as described earlier by Grimm and coworkers [17]. In brief, thyroid cells harvested from adherently growing confluent primary cultures were plated on agarose (1%) coated 96-multiwell test plates (Nunc, Wiesbaden, Germany) at a concentration of 4000 cells/200 µl Dulbecco's modified Eagle's medium (10% FCS) and incubated at 37°C and 5% CO_2. The medium was changed every second day.

Tumorigenicity

Animals and housing conditions: NMRI nude mice used for the in vivo tumor experiments were bred in our animal laboratory. Animals were fed ad libitum with a combined breeding/maintenance nude mice diet (Altromin, Germany) and water containing 1.3 g/l potassium sorbate (Merck, Darmstadt, Germany) and 2 g/l chloramphenicol (Sigma, Deisenhofen, Germany); the pH was adjusted to 2.5 with HCl. The animals were housed in macrolon cages (size II, Ehret, Germany) under specified pathogen-free conditions at 25°C, 70% relative humidity, and a 12 h/12 h light program.

Tumor implantation

Initially, 2×10^6 tumor cells in 0.1 ml phosphate-buffered saline (PBS) from monolayer cultures were inoculated subcutaneously into the right flank of 12 8-week-old NMRI (nu/nu) mice. They developed solid tumors within 2–3 months. For serial transplantation the solid tumors were cut into 2 mm^3 pieces and implanted with a trocar (13 G) into the right flank of another 20 8-week-old male mice. The tumor area was determined using planimetry. Parts of each solid tumor were excised for further characterizations.

Microscopy

Cells grown in plastic culture flasks (Greiner, Frickenhausen, Germany) and multicellular tumor spheroids were examined by phase contrast microscopy (Olympus-Microoptic, Unterhaching, Germany). The stained cells were observed by light microscopy for both histochemical and immunohistochemical analysis (Olympus-Microoptic). After labeling with fluorescent dyes (FITC, rhodamine) single cells were investigated either by fluorescent microscopy or by confocal microscopy (Leitz, Wetzlar, Germany).

Histological staining

For histological analysis of the recurrent tumor the xenograft tumors and the monolayer cells were stained with hematoxylin and eosin.

Immunohistochemical staining

For immunohistochemical staining of monolayers 2000 cells were seeded out into one chamber of a four-chamber Biocoat culture slide (Falcon, Heidelberg, Germany) and grown for 4 days. Subsequently the monolayers were washed twice in PBS, fixed with methanol and ethanol (1:1) or acetone, and treated with antibodies at optimal concentrations. Monoclonal anti-cytokeratin antibodies had been purchased from Immunotech (Dianova, Germany), anti-thyroglobulin antibodies from Dakopatts (Hamburg, Germany), anti-vimentin from Progen (Heidelberg, Germany), anti-fibronectin from Roche and anti-chondroitin-sulfate from Sigma. Antigen-antibody complexes were visualized with the indirect peroxidase technique [18]. Incubation with the first antibody was followed by incubation with the second antibody. After repeated washing with PBS and exposure to diaminobenzidine (Sigma) the specimens were dehydrated and embedded with entellan (Merck) and covered with glass slides.

Automatic image analysis

Morphometry including automatic image analysis was applied to quantitatively assess positive cells using a computer-assisted image analysis (Olympus Optical, Hamburg, Germany). Details and application of the method have been described by Grimm et al. [19].

Flow cytometric analysis of DNA and antigens

For the flow-cytometric determination of cellular antigens mouse monoclonal antibodies against cytokeratin, vimentin, and thyroglobulin were purchased from DAKO (Denmark). In each test antibodies were added to 10^5 cells, which had been prepared and fixed in ethanol (70%) exactly as previously described [17]. The cells were incubated for 60 min at room temperature in darkness and washed three times with PBS containing 2% FCS. The cells treated with unconjugated antibodies were again incubated for 45 min at room temperature with FITC-conjugated anti-mouse-IgG or swine-anti-rabbit-IgG antisera and washed afterwards. Prior to flow cytometric measurements the cells were treated for 60 min at 37°C with 50 mg RNase and 10 mg propidium iodide to obtain additional information about the DNA content. Finally, the cell suspensions were analyzed with a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany) equipped with an argon laser as described earlier [2]. Cells displaying fluorescence intensities above the upper limit of the negative control distribution were considered positive. Cell debris was gated out based on propidium iodide thresholds.

Analysis of electrophoretic mobility

The electrophoretic mobility, a marker for negative surface charge density, was analyzed by an Octopus free flow electrophoresis device equipped with an OCT-DET-VIS particle detection system (Dr. Weber, Kirchheim, Germany). The buffer film was pumped upwards at a speed of 1.4 mm/s through the separation chamber with a length of 500 mm, a width of 100 mm, and a thickness of 0.4 mm. Cells suspended in the separation medium at a concentration of 5×10^5 cells/ml were injected at the lower end of the chamber. The electrodes were separated from the eletrophoresis chamber by filter membranes and rinsed with media containing either 200 mM sodium acetate (anode) or 100 mM NaCl, 100 mM HCl, and 200 mM imidazol (cathode). The chamber medium was segmented, pumping simultaneously a central cell suspension medium containing 30 mM NaCl and 40 mM 2,2-bis (hydroxymethyl)-2,2',2''-nitrilotriethanol (BISTRIS), 20 mM *N*-(2-hydroxyethyl)piperazine-*N*'-(3-propanesulfonic acid) (EPPS), 170 mM sucrose, and 10 mM glucose (pH 7.4) between an anodal margin buffer containing 150 mM sodium sulfate, 40 mM BISTRIS, and 20 mM EPPS (pH 7.4) and a cathodal one containing 300 mM NaCl, 40 mM BISTRIS, 20 mM EPPS, and 75 mM sucrose (pH 7.4) through different nozzles at equal speed into the chamber [20, 21]. A voltage of 300 V was applied to this system at a current of 300 mA and 10°C.

In vitro hormone production of ML-1 cells

The quantities of thyroglobulin, fT3, and free thyroxine (fT4) released into the supernatant of one culture tube by 4.5×10^6 cells were determined using a radioimmunoassay developed by Brahms (Berlin, Germany). For this purpose supernatant was filled into tubes coated with antibodies specific to one of the antigens listed above. Subsequently a second antibody also specific to the antigen to be determined, but labeled with radioactive ^{125}I, was added. After incubation for 40 min at 37°C the supernatant was discarded, and the tube was washed three times with PBS. Then the radioactivity bound specifically to the walls of the tubes was determined by a gamma counter (Berthold, Nuremberg, Germany).

In vitro iodine and deoxyglucose incorporation

For determination of the glucose uptake capability of the thyroid cells 10^6 Bq ^{18}F-labeled deoxyglucose were added to 4.5×10^6 cells. After 20, 40, and 60 min of incubation at 37°C the cells were washed four times with PBS, and the radioactivity specifically bound to the cells was determined in a gamma counter. For determination of the iodine accumulation 4.5×10^6 cells were incubated at 37°C in the presence of 3×10^6 Bq ^{131}I and of various TSH concentrations (0, 40 and 200 mU/l). After 2, 8, and 24 h, the cells were washed four times with PBS, and the radioactivity specifically bound to the cells was determined in a gamma counter (Berthold; energy window: 364 keV ±10%). Incubation periods of 2, 8 and 24 h were chosen to assess a time-dependent effect and to confirm a long intracellular persistence of the radionuclide which is important for successful therapeutic strategies.

In vivo iodine and deoxyglucose incorporation

Diagnostic procedure of the patient

For identification of metastases within the body of the tumor donor patient, 2 weeks after removal of the tumor material used for culturing the cell line 3×10^8 Bq [^{18}F]-labeled deoxyglucose was injected intravenously. [^{18}F]-Labeled deoxyglucose positron-emission tomography was performed 60 min later to visualize glucose uptake in the tumor using a Siemens-CTI ECAT positron emission tomograph. After another 2 weeks 5×10^9 Bq ^{131}I was given orally. Then after another 3 days the radioactivity distribution was determined by the gamma camera Body-Scan (Siemens).

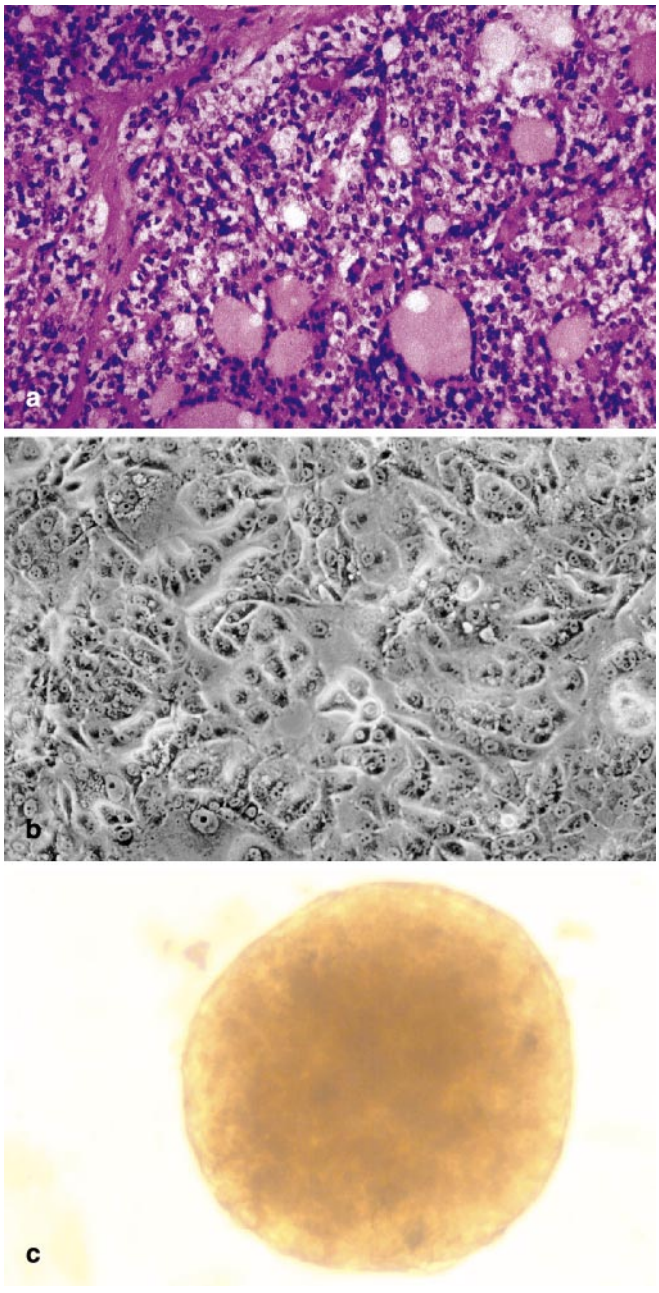


Fig. 1 **a** Hematoxylin-eosin stained section of the recurrent tumor used for establishing the cell line. ×200 **b** Monolayer. Phase contrast, ×200 **c** Multicellular tumor spheroid of the ML-1 cell line. Phase contrast, ×200

Experimental mouse investigation

To test the in vivo iodine uptake of ML-1 tumors growing in nude mice 1.2×10^4 Bq ^{123}I was administered orally to three animals. After 2 days the animals were killed. Whole-body radioiodine scans were performed using the gamma camera Basicam (Siemens). Scan time was 60 min.

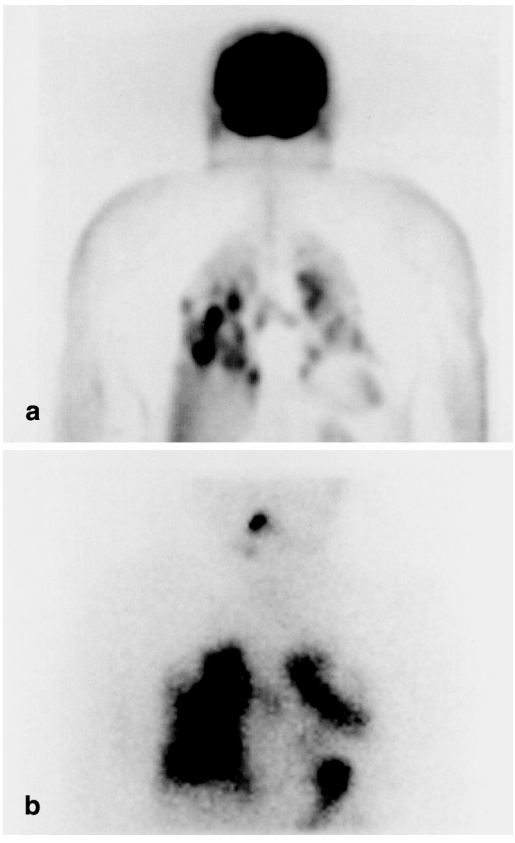


Fig. 2 Thorax ^{18}F-labeled deoxyglucose (**a**) and ^{131}I (**b**) scans after removal of the recurrent tumor of the ML-1 cell line from the patient. [^{18}F]-labeled deoxyglucose and ^{131}I were enriched in the lung metastasis

Results

Tumor origin

The cell line was derived from a tumor removed surgically from a 50-year-old woman. This tumor was a locally recurrent tumor which was still progressing despite previous surgery followed by two radioiodine treatments. It was classified according to the WHO International Histological Classification of thyroid tumors [1] as a poorly differentiated follicular thyroid carcinoma. It was staged according to the TNM [22] and graded as pT4N1M1 (pulmonary metastases). The tumor was slightly encapsulated showing follicular structures and areas of solid, papillary, growing cords (Fig. 1 A). At various sites it had already crossed the organ border or penetrated into blood or lymph vessels. Looking for cancer cells which may have remained during surgery in vivo ^{131}I and ^{18}F-labeled deoxyglucose incorporation tests were performed a few weeks after the recurrent tumor had been removed, which was the source of the ML-1 cell line. The examinations revealed that considerable quantities of glucose (Fig. 2 A) but small amounts of iodine (Fig. 2 B) were metabolized by pulmonary metastases, suggesting progressed dedifferentiation of the cancer cells growing within the donor patient.

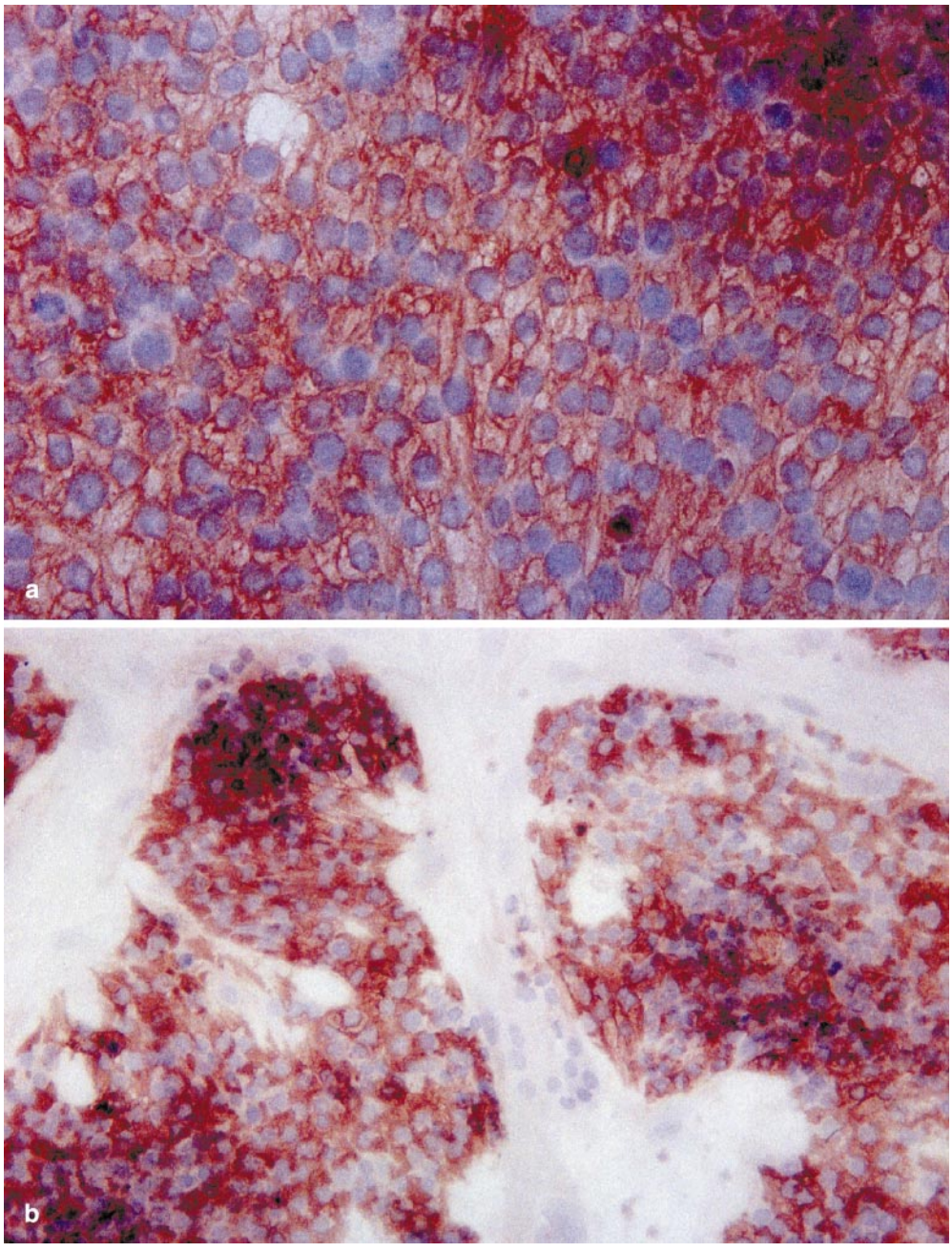


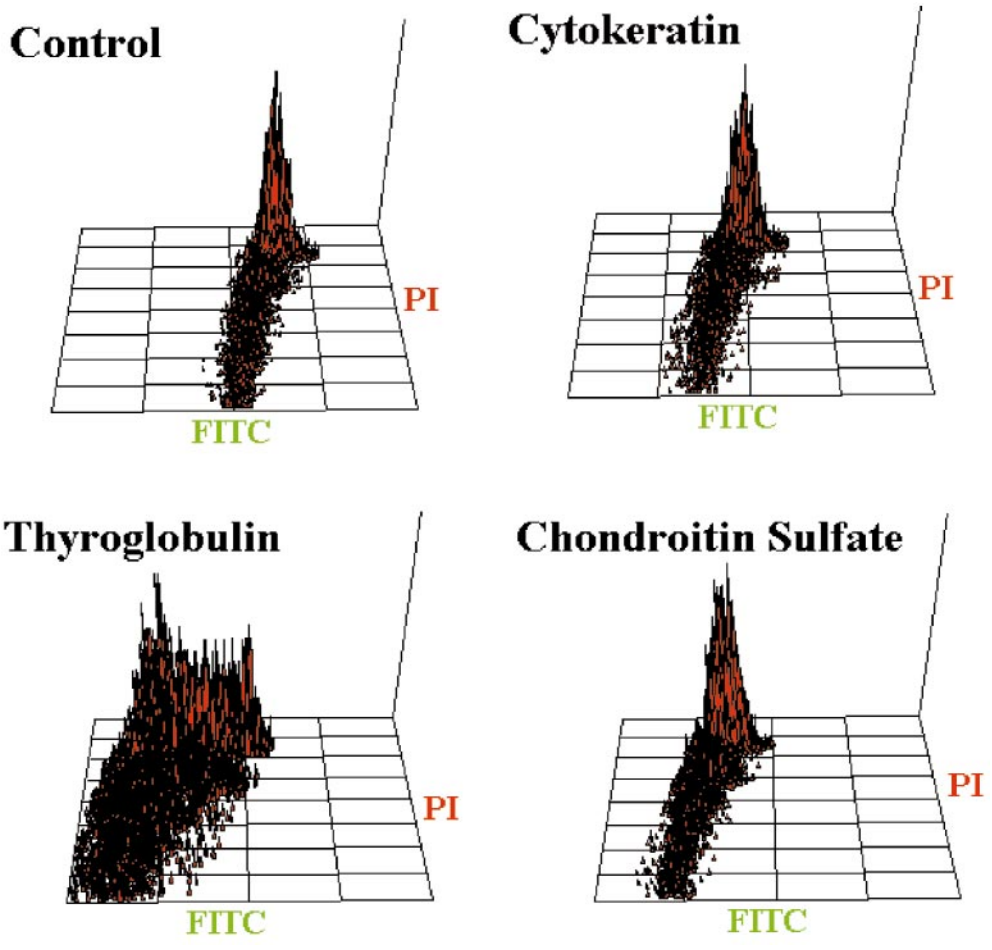
Fig. 3a,**b** Immunocytochemically stained ML-1 cells treated with anti-thyroglobulin (**a**, ×200) or with anti-cytokeratin (**b**, ×200) antibodies. **c** Dual-parameter flow cytometric analyses of cells of the ML-1 cell line after staining with propidium iodide and FITC-labeled anti-thyroglobulin (*top right*), anti-chondroitin sulfate (*bottom right*) and anti-cytokeratin (*bottom left*) antibodies. An IGG1 antibody was used as control (*top left*)

ML-1 cell line

The ML-1 cell line grew in monolayer cultures in plastic culture flasks (Fig. 1B). After 25 passages the cells still represented human follicular thyroid carcinoma cells, as at this time quantitative image analyses and flow cytometry revealed that 92%–95% of them had intracellularly accumulated thyroglobulin and chondroitin sulfate antigens (Fig. 3), and that 90% expressed vimentin. All cells were aneuploid with a DNA content of 2.6 c (Fig. 4). They formed multicellular tumor spheroids (Fig. 1C). When 4000 ML-1 cells were seeded above nonadherent agarose cushions, they formed multicellular tumor spheroids above virtually all of these cushions (liquid overlay technique). After 3 days of incubation tight regular aggregates of category III according to Carlsson et al. [23] were observed.

Furthermore, the ML-1cells were tumorigenous as demonstrated by the following xenograft transplantation experiments. Tumorigenicity was tested by transplantation of cell suspensions harvested from the ninth and tenth in vitro passage into NMRI nude mice. Between 6 and 8 weeks after subcutaneous inoculation of the tumor cells 10 of 12 animals developed palpable tumors in the region of the thoracic mammary fad pad (Fig. 5A). After 12 weeks the average tumor diameter was about 1 cm (Fig. 6). At this time a part of a solid tumor was excised, cut into 2 mm^3 pieces, and transplanted with a trocar into

Fig. 3c



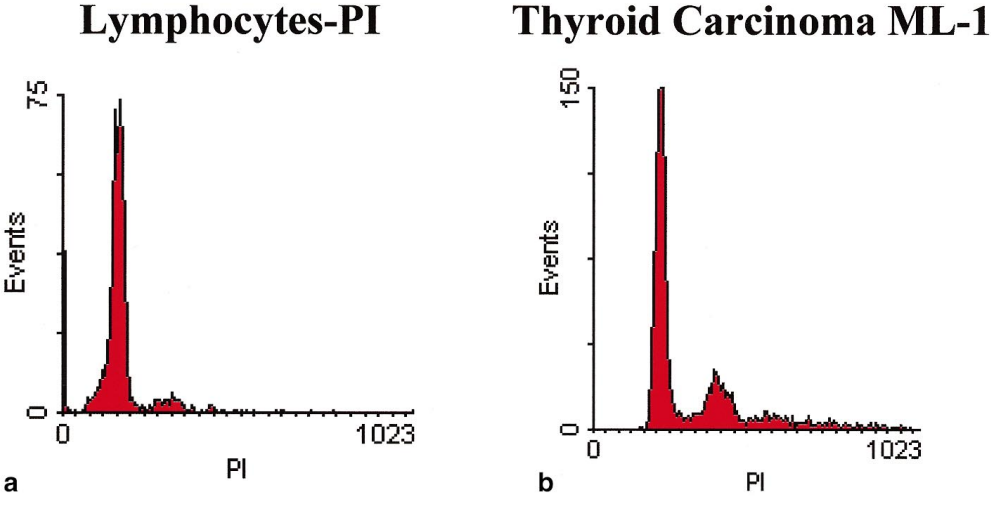


Fig. 4 Flow cytometric propidium iodide analysis of the DNA content of ML-1 cells (**b**) in comparison with diploid lymphocytes (**a**). *X-axis* Relative fluorescence intensity

another group of ten 8-week-old nude mice. After 3 months all the animals developed subcutaneous solid tumor areas ranging from 20 to 200 mm^2. The body weights of the animals did not decrease during the course of the experiment. Nine subsequent in vivo passages demonstrated that ML-1 is serially transplantable into nude mice (Fig. 6) and can therefore be used as a reproducible in vivo model. Even after the tenth passage of xenograft transplantations histology of the xenograft tumors revealed follicular structures and colloidlike areas (Fig. 5B). So far only tumors at the implantation site were observed in the nude mice, no metastases. However, these xenoplants incorporated ^{123}I (Fig. 5C).

Although the ML-1 cell line was quite homogeneous regarding the above characteristics above, cellular heterogeneity within the cell line was also maintained over

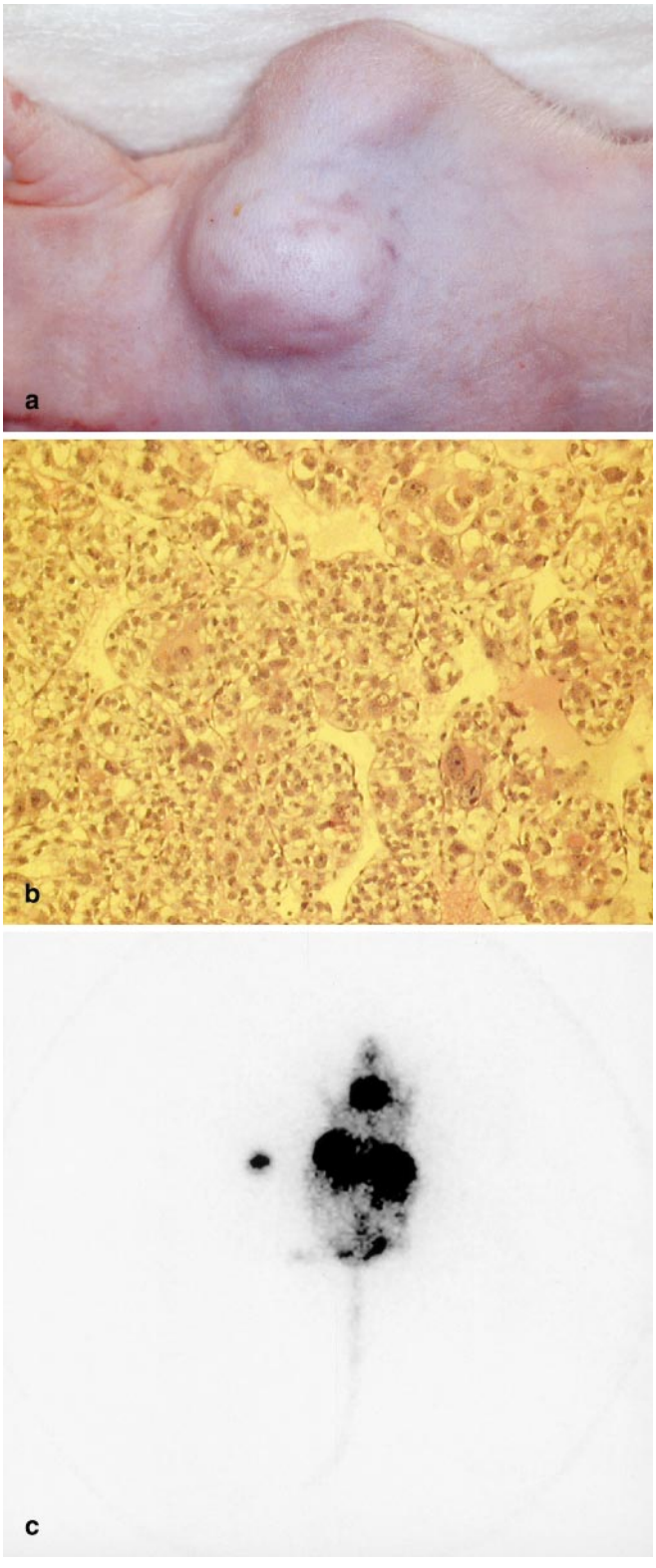


Fig. 5 **a** Photograph of an NMRI nude mouse bearing an ML-1 xenograft tumor. **b** Photograph showing a hematoxylin-eosin stained section of a xenotransplant ML-1 tumor obtained from a nude mouse after the tenth passage. Follicles can be seen. **c** ^{131}I scan of a nude mouse and its xenograft tumor which was removed and scanned (*left*)

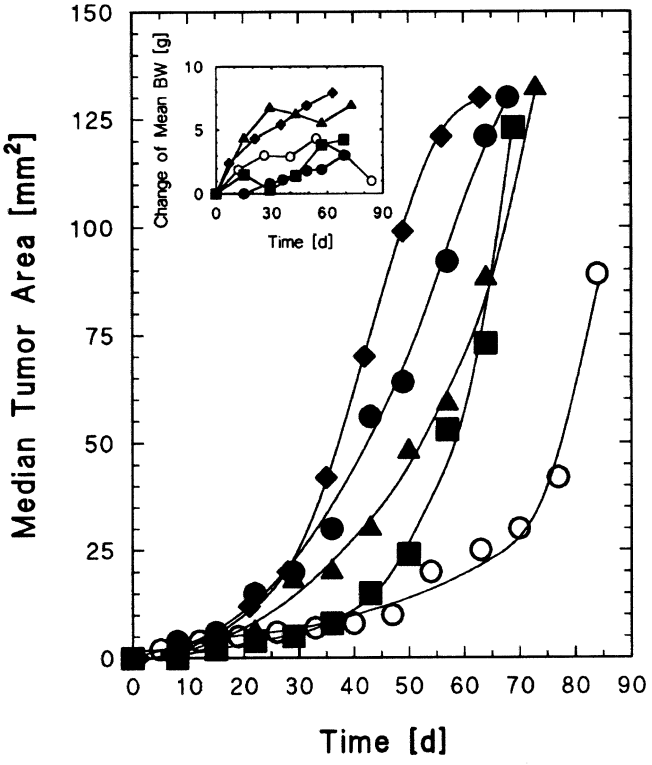


Fig. 6 Growth of the ML-1 cell line in nude mice. Increase in median tumor area in male NMRI (nu/nu) mice within 90 days. Tumor size after cell inoculation, during the first, third, fifth, and seventh passages is shown. *Insert* Simultaneous change in mean body weight (*BW*) during the course of the experiments. ○ Passage 0 (cell suspension); ▲ Passage 1; ■ Passage 3; ◆ Passage 5; ● Passage 7

25 passages. This was shown optically since a typical monolayer was composed of cuboid and elongated cells. Furthermore, only 72%–75% of the cells expressed cytokeratin (Fig. 3) and only 60–80% of the cells showed electrophoretic mobilities such as human erythrocytes (i.e., 1.1×10^{-4} cm^2 V^{-1} s^{-1}) while the remainder of the cells migrated faster.

Metabolism

In addition to the cellular features, very interesting and useful metabolic capabilities of the cell line were detected. With the help of immunohistological methods we found that the cells growing in monolayers secreted thyroglobulin and chondroitin sulfate as well as fibronectin into the interstitial space. Radioimmunoassays confirmed that the thyroid cells released thyroglobulin and also demonstrated the secretion of fT3. Within 3 days of culturing the thyroglobulin concentration within the supernatant rose to 600 ng/ml, and the fT3 content enhanced from a background concentration of 2 pM to a concentration of 6–7 pM fT3, while fT4 remained at the background level caused by the FCS. The cells incorporated deoxyglucose without prestimulation by TSH. Evaluated 20, 40 and 60 min after addition of [^{18}F]-labeled deoxy-

Table 1 Radioiodine-uptake of ML-1 cells in dependence on TSH concentrations and time of iodine presence (*cps* counts/s, n.d. not done)

Period of incubation	TSH concentration (mIU/l)		
	0	40	200
2 h	n.d.	61 cps	104 cps
8 h	n.d.	90 cps	140 cps
24 h	8 cps	401 cps	539 cps

glucose, 16%, 27%, and 34% of the radioactivity, respectively, was found to be specifically bound to the cells. The radionuclide ^{131}I was incorporated in small but reliably detectable amounts by the cells (Table 1). A 50- and 67-fold increase in radioactivity bound to the cells was measured when they had been incubated for 24 h in the presence of ^{131}I and 40 or 200 mIU/l TSH, respectively, compared to ML-1 cells incubated with equal quantities of ^{131}I in the absence of TSH (Table 1).

Discussion

In this study we established a human follicular thyroid carcinoma cell line which is tumorigenic in nude mice and very homogeneous regarding the expression of vimentin, thyroglobulin, and chondroitin sulfate antigens. This aneuploid cell line exerts a DNA content of 2.6 c, secretes constitutively fT3, takes up glucose permanently, and is capable of incorporating iodine upon stimulation by TSH.

The ML-1 cell line appears to represent human follicular thyroid carcinoma cells quite well because an aneuploid DNA content is rather common in cells of this type of tumor [24]. Also cytokeratin is considered to be a reliable marker of follicular thyroid carcinomas [25, 26]. Even the coexpression of cytokeratin and vimentin in thyroid tumors has been documented by several authors [27], although vimentin is an intermediate filament which normally appears in cells and tissue of mesenchymal origin but rarely in tumor tissues of epithelial origin [28]. The follicular thyroid tumor structures are found again when the cells formed subcutaneous tumors in nude mice after ten passages of in vitro growth.

The ML-1 cell line can be suggested as a tool of high reproducibility for both xenotransplantation experiments and future in vitro studies since it has proven to be quite stable. More than seven passages of serial subcutaneous transplantations in nude mice were possible, and even after 25 in vitro passages thyroglobulin and fT3 were released and iodine and glucose were still incorporated. Furthermore, the cell line had preserved homogeneity with regard to thyroglobulin, chondroitin sulfate, and vimentin antigen expression and also heterogeneity regarding cytokeratin, surface charges, and shapes of adhering cells. Although simultaneous double stains were not performed, the finding that more than 92% of the cells bear thyroglobulin antigens but not more than 75% were cytokeratin-positive suggests that, in addition to other thyroid cells, also those which contain thyroglobulin but do not express cytokeratin are present in this cell line. Such cells may, according to Böcker [29], be considered to be in a particularly dedifferentiated state and resistant against therapy.

The ML-1 cell line secretes constitutively fT3, which is a very important marker of proper thyroid function [30], while for some reason, which must still be investigated, fT4 cannot be detected in culture supernatants. Future experiments must be investigated whether fT4 is released after stimulation with TSH. It takes up glucose permanently, and depending on the presence of TSH it accumulates iodine over a period of 24 h, indicating that intracellular radioactivity would have reasonable time to destroy the cancer cells in therapy studies. This combination of features is currently unique for a permanent human follicular thyroid carcinoma cell line. It appears to render the ML-1 cell line a very valuable pharmacological test model for the considerable research which is currently going on with the aim of developing drugs for restimulation of iodine uptake of thyroid carcinoma cells [31] because, as explained above [3, 4, 5, 6, 7], the success of radioiodine therapy of thyroid tumors and especially of their metastases depends substantially on the iodine incorporation activities of the various cells forming the tumor.

As ML-1 cells can form spheroids, pharmacological tests may be expanded from studying drug effects on single cells or monolayers to investigating drug effects on three-dimensional cell aggregates simulating small tumors. Also, studies such as those on the coculturing of human thyroid carcinoma cells with human endothelial cell layers [2] can now be continued under more defined conditions than was possible when spheroids derived from various cancer patients had to be used. Despite the former donor-dependent variabilities we have previously confirmed that follicular thyroid carcinomas have a much greater effect on blood vessel endothelial cell monolayers than benign follicular adenomas or papillary carcinomas do [2]. Questions may now be addressed such as why the follicular carcinomas but not papillary thyroid carcinomas show mainly vascular dissemination, and signals triggered and exchanged at the contact sites can be studied exactly together with intercellular adhesion molecules possibly involved.

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