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Immunophenotypic and Molecular Cytogenetic Features of the Cell Line UP-LN1 Established from a Lymph Node Metastasis of a Poorly-differentiated Carcinoma

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Abstract. *A lymph node metastatic cell line (UP-LN1) was established in vitro from a poorly-differentiated epithelial tumor, and characterized for immunobiological and molecular cytogenetic profiles. The morphology of UP-LN1 cells when grown as monolayers was unique in that, apart from typical colonies of adherent epithelial cells, many loosely attached round cell colonies emerged on and around the patches of epithelial cells. By means of immunofluorescence/flow cytometric analysis, UP-LN1 cells showed selective expression of cytokeratin markers AE1 and CK20, but expressed AE3 and CK7 poorly. The expression of CEA and CK20 but not CK7 in UP-LN1 cells was also exhibited in the tumor biopsy by immunohistochemistry, suggesting a gastrointestinal origin of this tumor. Down-regulation of HLA-class I and other surface molecules including ICAM-1, CD44s, CD44v5, CD44v6 and E-cadherin were observed, which all pointed to a progressive/invasive phenotype of the tumor. Spectral karyotyping revealed a hypertriploid (~3n) chromosome content with 73-76 chromosomes, including numerical alterations and translocated derivative chromosomes. Seven different translocation chromosomes were observed, four of which involved chromosome 19. Numerical imbalances were also assessed by comparative genomic hybridization. This cell line should be useful for further studies of tumor invasion and metastatic processes because of the unusual in vitro and in vivo immunobiological and genetic characteristics observed.*

Over the years, significant advances have been made in the treatment of primary tumors, with corresponding improvements in life expectancy and quality of life for the patients. However, cases with spread or recurrent disease have a generally poor outcome, and account for most of the mortality and morbidity of cancer. While these two aspects of tumor progression, invasion and metastasis, complicate our approach to cancer therapy, our next challenges are how to control or intervene in tumor progression. On the cellular level, the progression to the invasive and metastatic stages are defined by the ability of the tumor to breach normal tissue, to transmigrate to extracellular matrix and to establish a growing tumor entity within normal tissues. To this end, molecular cancer research has sought to define cell properties critical for invasion and metastasis.

Tumor acquisition, processing, cell culturing and cryopreservation programs have collectively become important in the oncology laboratory, and are used for both basic and therapeutic research, as well as patient monitoring in the course of treatment (1). Although many human carcinoma cell lines have been studied, most of these cell lines were derived from primary tumors. Only a few cell lines from lymph node metastatic sites are available for studies of tumor progression, invasion and the metastatic cascade (2-4). The first step of tumor spread is to regional draining lymph nodes. To control this early step of invasion by biological and immunological interventions is important from both basic and

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Key Words: Poorly-differentiated carcinoma, lymph node, metastasis, cell line, SKY, CGH.

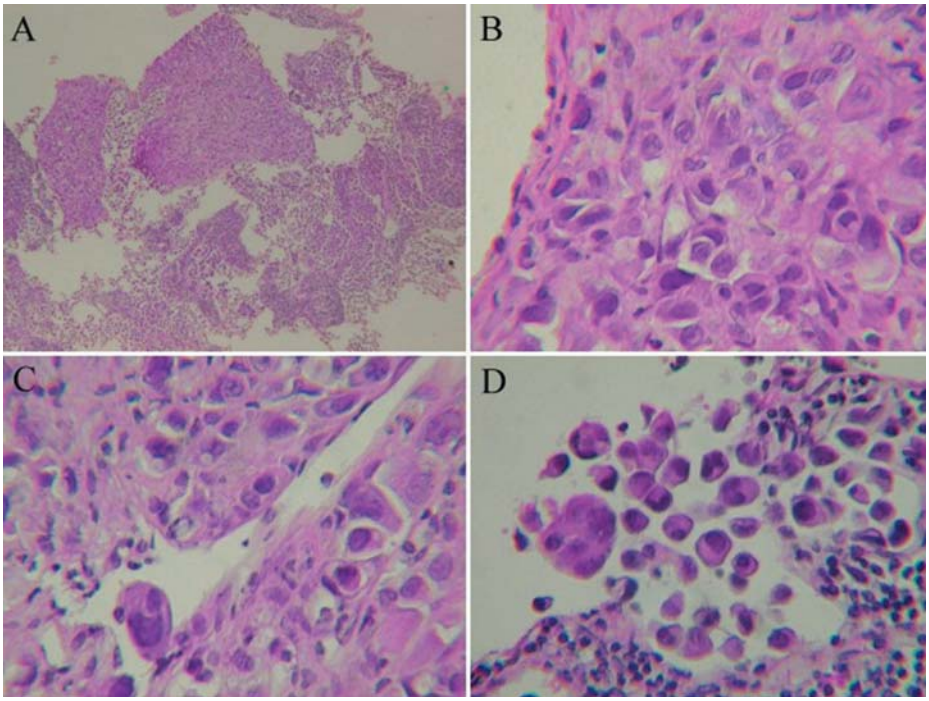

Figure 1. *H&E staining of the original tumor biopsy from which the UP-LN1 cell line was derived. The lymph node structure shows destroyed integrity (A) in low magnification (x 40). Solid tumor areas consisting of compact epithelial tumor cells of poorly-differentiated phenotype are seen in (B) and (C). Single tumor cells and small groups of round tumor cells located within areas of lymphoid cells or blood vessels are noted in (C) and (D).*

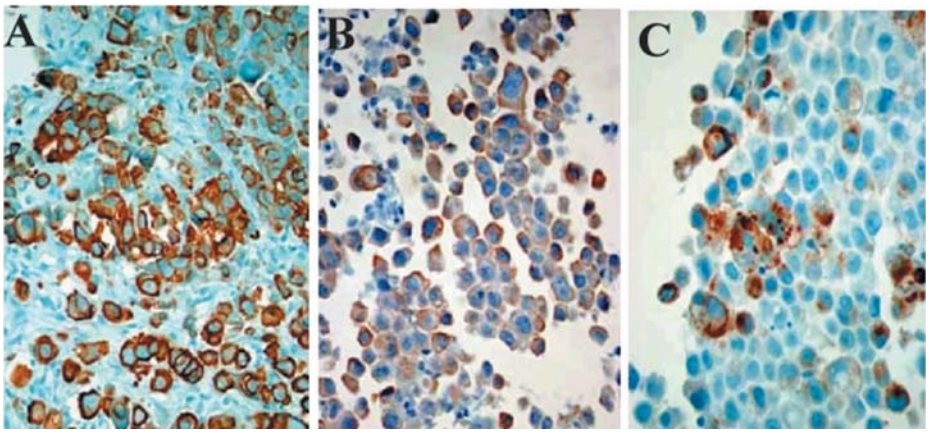

Figure 2. *Immunostaining of paraffin sections from the original biopsy from which the UP-LN1 cell line was derived. Positive cytoplasmic staining (brown) is seen for pan-cytokeratin marker MNF116 (A), cytokeratin 20 - CK 20 (B) and carcinoembryonic antigen - CEA (C), supporting that the tumor is epithelial, probably of gastrointestinal origin.*

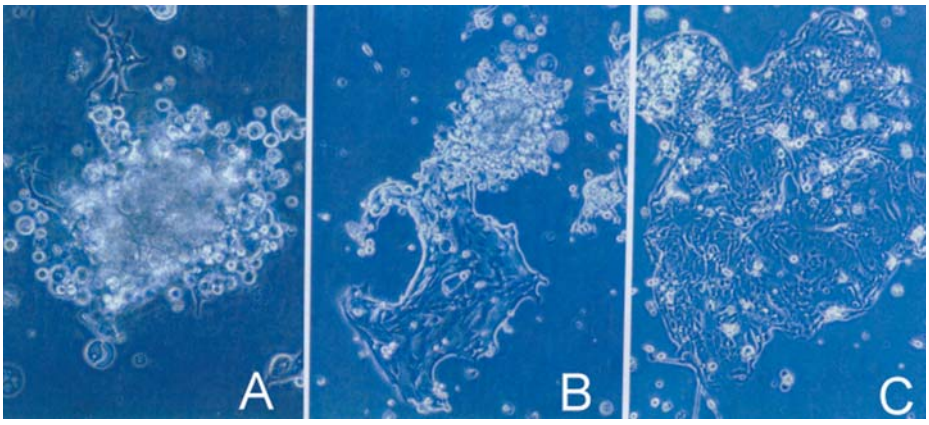

Figure 3. *Representative in vitro growth pattern of cultured UP-LN1 cells. In (A) and (B), colonies of adherent and compact epithelial tumor cells are seen. In (B) and (C), semi-adherent aggregates (colonies) of round cells are located on the top or at the periphery of colonies of adherent and compact epithelial tumor cells.*

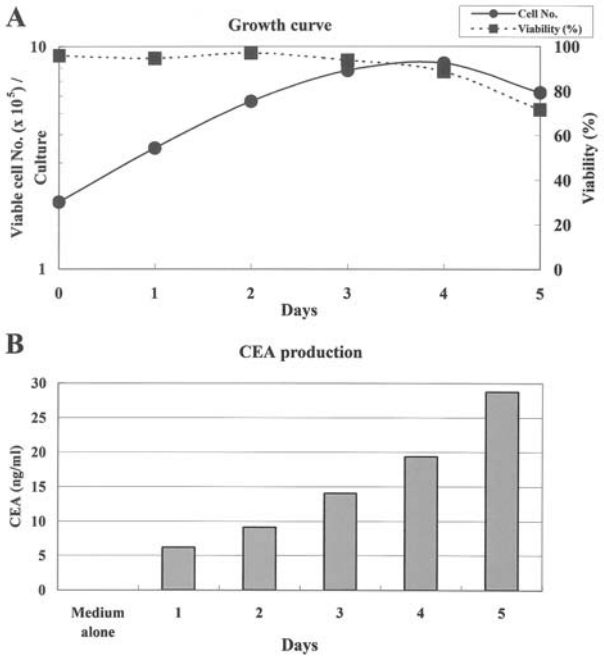

Figure 4. *Growth curve and production of carcinoembyonic antigen - CEA in the used medium of cultured UP-LN1 cells. The upper panel (A) shows a typical in vitro growth curve of UP-LN1 cells in which the total viable cell number/dish and % cell viability at each time point are indicated. In the lower panel (B), production of CEA in the used medium of cultured cells at each indicated time point is shown. Note that there was no CEA detectable in fresh culture medium.*

therapeutic points of view. In the past few years, we have, therefore, directed our efforts to the establishment and maintenance of such metastatic cell lines. Here, we describe the establishment and initial characterization of a new cell line, UP-LN1, which was derived from a lymph node metastasis of a poorly-differentiated epithelial carcinoma.

Materials and Methods

Patient history and tumor specimens. A male patient in his late sixties was admitted to Chang Gung Memorial Hospital, Taoyuan, Taiwan, with chief complaints of masses at the neck. Three weeks after the hospital admission, the patient died, most probably due to metastatic disease. At the initial physical examination, several lymph nodes were noted in the right and left neck regions. X-ray analyses of the chest area revealed no further tissue masses in addition to those in the neck area. A sonographically-guided core-needle biopsy (5) of an enlarged lymph node in the right supraclavicular fossa was performed by one of the authors (AS-BC), using an 18-G needle. Three tissue pieces of the jelly-like specimens measuring from 0.4 x 0.1 x 0.1 cm to 0.8 x 0.1 x 0.1 cm were removed, embedded in paraffin and subjected to histopathological examination and immunochemical analysis. The remaining portion of the biopsy specimen was sent to the tumor immunology laboratory for tissue processing, cell culture and cryopreservation.

Table I. *Expression of selected surface and/or cytoplasmic antigens by UP-LN1 cells, as determined by immunofluorescence/flow cytometric analyses.*

Antigen expression	% positive cells (relative fluorescence intensity)
Surface antigen	
HLA-A,B,C	66.1 (101.1)
HLA-DR	1.1
HLA-G	1.3
ICAM-1	8.0
EMA	12.2 (94.4)
Epi-CAM (40-kDa)	96.9 (274.8)
Le^y	3.2
Sialyl-Tn	16.0 (32.6)
CD44s	58.4 (50.5)
CD44v5	3.7
CD44v6	15.8 (57.8)
E-cadherin	13.9 (30.2)
EGFR (R1)	2.0
CEA	52.7 (160.7)
Cytoplasmic antigen	
AE1	91.1 (138.7)
AE3	13.6 (33.9)
AE1/AE3	90.0 (57.2)
CK-7	0.6
CK-20	83.1 (89.9)
MAK-6[a]	84.2 (83.3)
EMA	39.7 (33.2)
Epi-CAM (40-kDa)	93.5 (274.8)
Le^y	36.8 (20.1)
Sialyl-Tn	20.3 (54.3)
CD44s	61.8 (28.8)
CD44v5	62.1 (13.4)
CD44v6	0.3
E-cadherin	14.3 (21.1)
Bcl-2	5.4
CEA	61.5 (50.9)

[a]Detected by a mixture of two MAbs: KA-4 to cytoplasmic cytokeratins types 14, 15, 16 and 19, and UCD/PR-110.11 to two cytokeratins types 8 and 18.

Cell line establishment and culture conditions. For the establishment of the UP-LN1 cell line, a single cell suspension was prepared from the tumor tissue by mechanical mincing. Cells were seeded in dishes with 56.7 cm^2 culture area (Nunc, Rockilde, Denmark) and cultured at 37˚C in a humidified atmosphere containing 5% CO_2 and 95% air. A medium previously optimized from the growth of other malignant tumors was used, consisting of RPMI-1640 supplemented with 2 mM L-glutamine, 10 mM HEPES buffer, 10% heat-inactivated fetal bovine serum (FBS), penicillin (50 U/ml) and streptomycin (50 μg/ml). When the cultures were subjected to subculturing, cells were pooled from both floating and attached cells dissociated by trypsinization and, after washing in complete growth medium, the cells were expanded to T-25 flasks or dishes. Intermittently, cells in some cultures were frozen in 90% FBS and 10% dimethyl sulfoxide in liquid nitrogen. Growth curves, each covering a total of five days of culturing without change of medium, were constructed, from which the population doubling time and

Table II. *Data from SKY, CGH and STR analyses of the UP-LN1 cell line.*

Analysis	Findings
CGH	Gains: 1p34-pter, 1p21-qter, 3q21-27, 5p14-pter, 11p-q22, 12p11-q13, 12q23-qter, 14q23-qter, 16, 17, 20q Losses: 2, 3p14-cen, 4p16, 4q13-qter, 10p12-pter, 18, 21, Xp11-q22, Xq21-qter
SKY	73-76<3n>,XX,-Y,del(3)(p11p14),del(4)(q31),del(5)(q14),der(5)del(5)(q31)t(5;11)(p11;q11),-6,der(6)t(6;19)(q23;p?),-8,der(10)t(10;19)(p11;p?),+11,+11,der(11)t(11;20)(?;?),del(12)(q15),der(13)t(12;13)(q13;p13),der(15)(10q23-qter::15p11.2)x2,+16,-18,del(18)(q21.2),-19,der(19)t(15;19)(?;?),der(19)t(19;22)(?;?),+20,+22[cp10].
STR	Amelnogenin (allele size 34) D3S1358 (15), FGA (23), D5S818 (13), D7S820 (9,11), D8S1179 (13), vWA (14,17), D13S317 (13,25), D18S851 (13,16), and D21S11 (29,31)

saturation density were determined. For each time point, triplicate culture dishes were removed from the incubator for cell counts (viable and non viable cells) and the used media were harvested for CEA concentrations by an immunoassay described below.

Immunohistochemistry. Immunochemical analyses of the paraffin sections were performed for the following markers: prostate specific antigen (PSA), prostate specific acid phosphatase (PSAP) (6), carcinoembryonic antigen (CEA), pan-cytokeratin (MNF116) (7), cytokeratin 7 (CK7) and cytokeratin 20 (CK20), using automated methodology (Enhanced DAB paraffin, IHC Staining Module, Ventana Medial Systems, AZ, USA).

Immunoassay for CEA. The ARCHITECT CEA assay was used, which is a two-step immunoassay to determine the presence of CEA in serum or medium, using the Chemiluminescent Microparticle immunoassay (CMIA) technology (Abbott Laboratories, Abbott Park, IL, USA). In the first step, sample and anti-CEA-coated paramagnetic microparticles were combined, whereby CEA present in the sample binds to the anti-CEA-coated microparticles. After washing, anti-CEA acridium-labelled conjugate was added to the second step. Pretrigger and trigger solutions were then added to the reaction mixture. The resulting chemiluminescent reaction was measured as relative light units. The assay protocol allowed detection within the range from 0 – 1500 ng/ml.

Tumorigenicity in immunodeficient mice. Tumorigenicity assay of UP-LN1 was carried out in mice with severe combined immunodeficiency (SCID) (n=4). The analyses were conducted upon approval from the Animal Ethics Committee, Chang Gung University, Taiwan. Six-week-old female SCID mice (CB.17) were purchased from the Central Animal Facility of the National Taiwan University Hospital, Taipei. Floating cells and easily detached cells were collected in a tube, while attached cells were collected separately by trypsination and washing once in phosphate-buffered saline (PBS). The two populations of cells were pooled, mixed in PBS and counted. UP-LN1 cells (5×10^6 /0.1 ml PBS) were injected subcutaneously at a site above the hind leg of each mouse. The animals were examined every 2 days for a period of 103 days to estimate the growth of tumors. The volume of palpable tumor nodules was estimated according to the formula: volume (mm^3) = $0.4 \times a \times b^2$, where (a) is the major tumor diameter and (b) is the minor diameter perpendicular to the major one (8)

Determination of nuclear DNA content by flow cytometry. The DNA contents of nuclei from cultured UP-LN1 cells and normal adult peripheral blood mononuclear cells (PBMCs) were analyzed according to the method described previously (9). The DNA index relative to normal PBMCs was calculated.

Monoclonal antibodies for immunophenotyping of UP-LN1 cells. A panel of monoclonal antibodies (MAbs) were used for immunophenotyping of the UP-LN1 cells. These included MAbs for antigen detection of HLA-A,B,C (W6/32, manufactured by Dako, Glostrup, Denmark), HLA-DR (DR22, Dako), HLA-G (MEM-G/1, Serotec, Oxford, UK), Lewis Y (Le^y) (ABL 364, Dr. Loibner, Sandoz, Vienna, Austria) (10), ICAM-1 (6.5B5, Dako), CD44 (SFF-22, BenderMed System, Vienna, Austria), CD44v5 (VFF8, BenderMed System), CD44v6 (VFF9, BenderMed System), EGFR (R1, Santa Cruz Biotechnology, Santa Cruz, CA, USA), AE1 (AE1), AE3 (AE3), AE1/AE3 (mixture of MAbs referred to above), CK7 (OV-TL 12/30, Dako), CK20 (KS20.8), MAK-6 (mixture of two MAbs to cytokeratins including types 14, 15. 16. 19 for one MAb and type 8 and 18 for another MAb, Zymed, San Francisco, CA, USA), EMA (epithelial membrane antigen, E29, Dako), 40-kDa Epi-CAM (MAC-CO1) (11), Sialyl-Tn (49-H8, Dr. Longenecker Biomira, Edmonton, Canada) (12), E-cadherin (67A4, Biodesign, Kennebunk, ME, USA), bcl-2 (124, Dako) and CEA (Novacastra, Newcastle, UK).

Surface/cytoplasmatic immunofluorescence and flow cytometric analysis. Monodispersed cells harvested from cell cultures were dispensed into test tubes (5×10^6 cell /ml PBS/tube) and centrifuged at 400 x g to pellet the cells. Test antibodies (5 μg/ml) were added (100 μl/tube) and the mixture incubated at 4˚C for 30 minutes. Immunofluorescence-based detection of surface antigen (use of unfixed live cells) was performed with various antibodies, including appropriate positive and negative controls, followed by flow cytometric analysis using methods described previously (13). For cytoplasmic antigens, the cell pellets were first treated with 1% paraformaldehyde at 4˚C for 20 minutes and then with cold acetone for 3 minutes (14). This allowed the immunoreagents and washing solution to penetrate through the cell membrane. Positive (anti-HLA-A,B,C, W6/32) and negative (PBS in place of primary antibody, isotype-matched irrelevant monoclonal antibody, or polyclonal goat anti-mouse IgG antibodies with prior addition of test primary antibody) controls were included in each test.

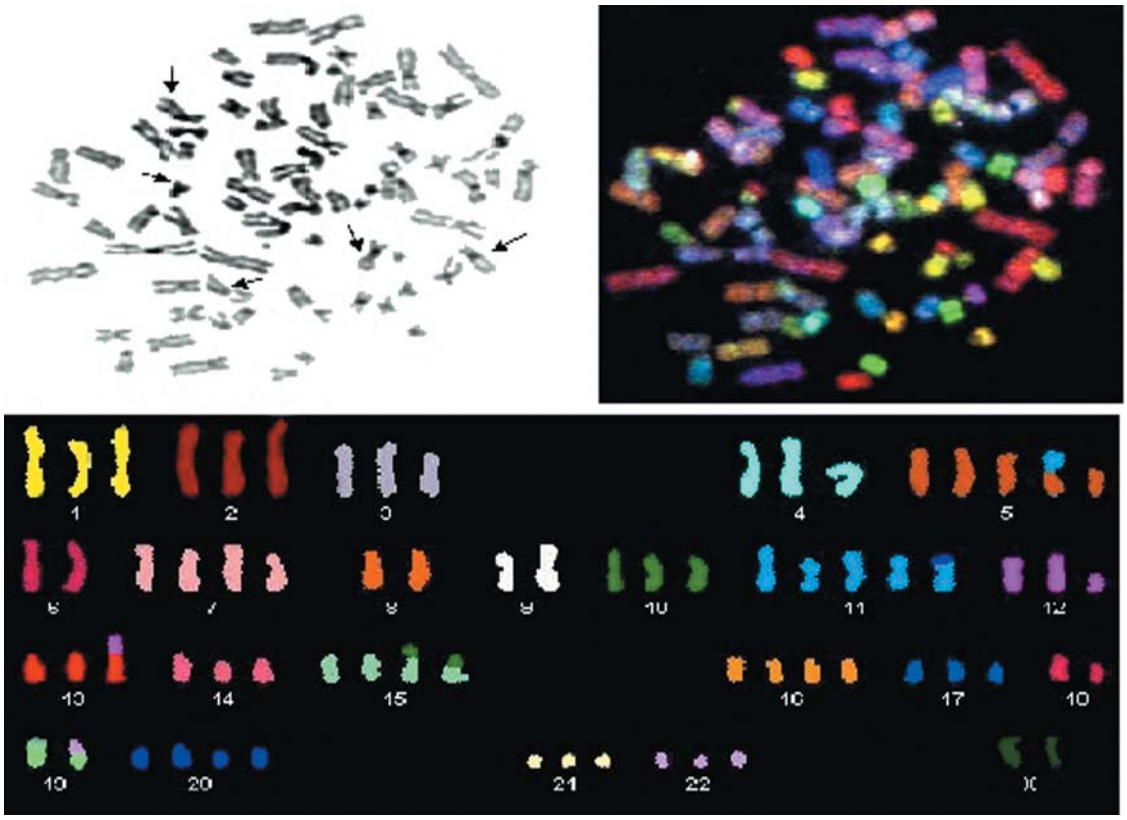

Figure 5. *SKY analysis of UP-LN1 cells. A representative metaphase spread is shown in three formats: at the top as an inverted DAPI banding image (upper left panel) where detectable chromosomal translocations are marked by arrows; after SKY hybridization (upper right panel); and with chromosomes arranged in a SKY and presented in classified pseudo-colors (lower panel).*

Comparative genomic hybridization (CGH) and digital image analysis. High molecular weight DNA was extracted from UP-LN1 cells using standard methods. The cell line DNA was directly labelled by nick-translation using FITC-12-dUTP (DuPont NEN, Boston, MA, USA). The nick-translation conditions were adjusted to obtain DNA fragments ranging from 300 to 2000 bp in size. Spectrum Red-labelled normal DNA (Vysis, Downers Grove, IL, USA) was used as reference in the CGH hybridisation. The cell line DNA, normal reference DNA and unlabelled Cot-1 DNA (Life Technologies, Gaithersburg, MD, USA) were mixed together with 10 μl of hybridization buffer (formamide 50%, dextran sulfate 10% in 2 x SSC), denatured and applied onto denatured normal metaphase slides (Vysis). Hybridization was performed at 37˚C for 72 hours. After post-hybridization washing and drying, the slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) (Sigma, Saint Louis, MO, USA) 0.1 μg/ml, in an antifade solution, Vectashield (Vector, Burlingame, CA, USA). A control experiment was performed where normal male DNA was hybridized against normal female DNA. Fifteen three-color digital images (DAPI, FITC and Spectrum Red fluorescence) were captured using a Zeiss Axioplan 2 imaging (Carl Zeiss Jena GmbH, Jena, Germany) epifluorescence microscope and analyzed with the isis/CGH software (Metasystems). At least 12 ratio profiles were averaged for each chromosome to reduce noise. The chromosomal regions were interpreted as lost when the green-to-red ratio was less than 0.8, and as gained if the ratio was greater than 1.2. A high-level amplification was defined if the ratio exceeded 2.0. Heterochromatic regions, the short arm of the acrocentric chromosomes and chromosome Y were excluded from the evaluation. Similarly, chromosomes 19 and 22 were excluded from the analysis, to avoid false-positive results due to GC-rich regions in these chromosomes.

Chromosome preparation, G-banding and spectral karyotyping (SKY). UP-LN1 cells were grown in culture under the conditions described above. After cell harvesting, metaphase chromosomes were prepared and used for G-banding and SKY analysis. A total of ten metaphases were analyzed after SKY and G-banding, respectively. The clonality criteria and the description of karyotypes followed the recommendations of the ISCN 1995 (15).

SKY was performed according to the protocol recommended by the manufacturer (Applied Spectral Imaging, ASI, Migdal Haemek, Israel). Image acquisitions were performed using a SD200 Spectracube system (ASI) mounted on a Zeiss Axioskop microscope with a custom-designed optical filter (SKY-1, Chroma Technology, Brattleboro, VT, USA).

Short tandem repeat (STR) profiling. DNA extracted from UP-LN1 cells was genotyped using the AmpFLSTR Profiler Plus™ PCR amplification kit and an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA), according to the protocol

recommended by the manufacturer. Ten STR markers were thus analyzed including D3S1358, FGA, D5S818, D7S820, D8S1179, vWA, D13S317, D18S851, D21S11 and Amelnogenin.

Results

Histopathology of the tumor biopsy. The tumor tissue sections were examined by light microscopy after H&E staining, which revealed a poorly-differentiated carcinoma. Representative microphotographs are shown in Figure 1. The histopathological examination revealed cores of lymphoid tissue with metastatic, poorly-differentiated carcinoma cells. The architecture of the affected lymph nodes was largely destroyed and the lymphoid tissue mostly replaced by tumor cells. Interestingly, both solid tumor areas of compactly packed tumor cells, and singly distributed tumor cells, with occasional groups of 2-6 tumor cells in small blood vessels and inter-lymphoid cell areas, were observed (Figure 1). Immunostaining revealed positivity for CEA, MNF116 and CK20 (Figure 2), while CK7, PSA and PAP were negative. Taken together, the clinical, histopathological and immunostaining findings suggested that the lymph node metastasis was derived from a poorly-differentiated carcinoma, possibly of gastrointestinal origin.

Establishment of the UP-LN1 cell line and growth pattern in vitro. During the first two weeks of the primary culture, fibroblast growth was found on many areas of the bottom of the flask. Some smaller epithelial colonies also emerged together with loosely attached round cells growing as clusters or aggregates either on the top of fibroblastic monolayers or on epithelial colonies. Such cell clusters were also found independently on areas other than fibroblastic and epithelial monolayers. As the passages continued, some cultures were deliberately set up by those loosely attached round cells by gentle washing with complete growth medium, while some cultures were set up by cells removed by light trypsinization (short-term exposure to EDTA-trypsin solution). The latter procedure tended to favor the removal of epithelial cells instead of the more firmly attached fibroblastic cells. After using such a selective detachment process of epithelial cells in the following three *in vitro* passages, a fibroblast-free epithelial cell population was finally obtained. During the cultures containing mostly loosely attached cells, fibroblasts were no longer seen. Interestingly, in these cultures both epithelial colonies as well as aggregates of loosely attached cells were both present. This cell population and the culture population of loosely attached cells were pooled and the mixture was allowed to grow *in vitro* continuously.

The representative morphology of UP-LN1 live cells in monolayer culture is depicted in Figure 3. This shows a colony appearing as an aggregate of semi-attached and contact-inhibited round cells (Figure 3A). The three dendritic cells at the upper left of the frame (Figure 3A) were capable of growing to form a large colony, as shown in the lower part of Figure 3B. Two different kinds of colonies are also observed in Figure 3B, one consisting of many attached epithelial cells and the other being an aggregate of loosely attached round cells. In Figure 3C, one or two irregular-shaped colonies are illustrated, in which some round cells adherent to the small-sized colony are noted. When a few dendritic cells as groups started to proliferate and form colonies, they became more compact among epithelial cells and lost the individual dendritic morphology within each colony.

In vitro growth curve of and production of CEA by UP-LN1 cells. Growth curves were constructed from two independent experiments with cells at the 4th and 17th *in vitro* passages, and identical results were obtained. The results are exemplified for passage 17th in Figure 4A. *In vitro* growth of the UP-LN1 cell line was characterized by an estimated doubling time of 36 hours and a saturation density of 9.1 x $10^4/cm^2$. The progressive production of CEA in the used medium by the cultured cells was also clearly demonstrated (Figure 4B).

Xenotransplantability of UP-LN1 cells in SCID mice. Four out of four SCID mice developed solid tumors at the injection sites after subcutaneous inoculation with 5 x 10^6 UP-LN1 cells. Two and a half months after the inoculations, the average size of tumor was 1,964 mm^3, in agreement with the malignant nature of the UP-LN1 cells. Cultured cells established from the xenograft behaved similarly to the original UP-LN1 cells regarding *in vitro* morphology and growth pattern, however appeared to develop more aggregates of round cells during the earlier passages as compared to the original UP-LN1 cells.

Expression of immunophenotypic markers. A panel of surface and cytosolic antigen markers were evaluated for expression in UP-LN1 cells using indirect immunofluorescence and flow cytometric analysis (Table I). While some of the cytokeratin markers tested, such as AE1, CK20 and MAK-6, were positive, others, including AE-3 and CK7, were either negative or only weakly expressed. We also noted that HLA-class I molecules were expressed by only 66% of UP-LN1 cells with a rather low relative fluorescence intensity. Moreover, the UP-LN1 cells expressed a rather low level of surface ICAM-1. The surface 40-kDa Epi-CAM was expressed abundantly, while other surface pan-carcinoma antigens, such as EMA, Le^y, and sialyl-Tn and E-cadherin, were clearly down-regulated.

DNA content. Analysis of DNA from UP-LN1 cells (at the 4th and 6th passage levels) by flow cytometry indicated that the samples contained one major aneuploid population of cells with a DNA index of 1.7 relative to the DNA content

of normal human diploid peripheral blood mononuclear cells. These findings suggest that the UP-LN1 cell line has a hypertriploid DNA content.

Genetic characterization of the UP-LN1 cell line. The results from analyses by CGH, SKY and STR profiling are summarized in Table II. The chromosomal composition of the UP-LN1 cell line was determined by the combined use of SKY and G-banding analyses. The SKY results are illustrated for a representative metaphase in Figure 5. All metaphases analyzed displayed a hypertriploid chromosome content with the composite karyotype 73～76 <3n>, which is in line with the DNA index determined by flow cytometry. The karyotypic analysis of UP-LN1 cells revealed few numerical and structural abnormalities, which were represented in two or more metaphases (Table II; Figure 5). Seven different translocation chromosomes were observed, four of which involved chromosome 19. The karyotypic analyses were also complemented with CGH analyses to detect numerical imbalances in the UP-LN1 cells (Table II).

Copy number gains detected for 5p14-pter, 11p-q22. 12p11-q13, 12q23-qter and 20q by CGH corresponded well to the karyotypic abnormalities of der(5)del(5)(q31)t(5;11) (p11;q11), +11, +11, der(11)t(11;20)(?;?), der(13)t(12;13) (q13;p13) and +20. Similarly, the losses of 3p14-cen, 4q13-qter, 10p12-pter and 18 revealed by CGH were consistent with the karyotypic alterations of del(3)(p11p14),del(4)(q31),der(10)t(10;19)(p11;p?),der(15)(10q23→qter::15p11.2)x2,-18 and del(18)(q21.2). Thus, the CGH imbalances detected in this cell line were in accordance with the karyotypes determined by SKY and G-banding.

Discussion

In this study, we described the establishment and initial characterization, with respect to immunobiological and molecular cytogenetic profiles, of the UP-LN1 cell line derived from a poorly differentiated lymph node metastasis. This cell line exhibited a slightly different growth pattern and morphology *in vitro* as compared to other carcinoma cell lines maintained in our laboratory, which were derived from primary tumors, ascites or bone marrow metastases of head and neck cancers and nasopharyngeal carcinomas. In our experience, most of the carcinoma cell lines displayed typical epithelial cell morphology with a property of firm attachment onto the substratum of the culture flasks. In contrast, UP-LN1 cells grew both as monolayer epithelial cells and as loosely attached round viable cells, usually appearing as clusters on or around the patches of epithelial cells. These round cells could also be seen floating in the culture supernatant or settled on the bottom of the flask at some distance from the epithelial cells. These three components (attached, loosely adherent and non-adherent cells) were all of epithelial type, since they each displayed the same characteristic phenotypic profiles, a few days after each of these cells was independently plated in a culture flask. Expansion of tumor epithelial cells to become completely confluent on the bottom of the whole culture plate was not seen, but instead they presented themselves as many independent colonies of round cells or epithelial tumor cells, and few floating single round cells. Colonies with a mixture of the former two cell types were also frequently observed. The round cell colonies displayed three-dimensional growth, showing loss of contact inhibition. When some of these colonies of either small or large size detached from the culture flask surface, these floating colonies appeared similar to multicell tumor cell spheroids (16, 17). It is not clear whether the semi-attached round cells observed *in vitro* originated from singly dispersed tumor cells between lymphoid cells *in vivo*. Similarly, it could not be determined whether the patches of attached epithelial tumor cells *in vitro* came from compact solid tumor cells observed *in vivo*. Despite the differences observed, preliminary results from cell culturing and cloning showed that both cell types seen *in vitro* were mutually convertible.

The UP-LN1 cell line and the original *in vivo* tumor both showed selective expression of cytokeratins. The coordinate expression of cytoplasmic CK7 and CK20 has provided a useful means for diagnostic purposes, particularly in situations where the primary tumor is unknown (18, 19). The discoordinate expression of these two cytokeratins have been previously applied to distinguish gastrointestinal tumors ($CK7^-/CK20^+$) from lung tumors ($CK7^+/CD20^-$), regardless of whether the tumor was of primary or metastatic origin (20). In this study, both the original tumor and the cultured tumor cells expressed pan-cytokeratin markers and CK20, while CK7 was negative, suggesting a gastrointestinal origin (Table I, Figure 2). Furthermore, the negative results for PSA and PSAP clearly excluded prostate cancer, while the positive expression of CEA supported that the primary tumor was of gastrointestinal origin.

The strong expression of the pan-carcinoma-associated surface antigen (40-kDa Epi-CAM) (11) by UP-LN1 supports that the original tumor was of epithelial origin. On the other hand, two other carcinoma-related surface antigens, Le^y (21, 22) and Sialyl-Tn (12, 23), that have been used as therapeutic targets for small cell lung carcinoma and breast carcinoma in clinical studies, were not abundantly expressed by the UP-LN1 cells (<16%). This would clearly exclude the possibility of using these two targets for therapeutic purposes for patients such as the one bearing the metastatic UP-LN1 tumor. It is of interest to observe the down-regulation of both surface HLA-class I and ICAM-1 antigens in UP-LN1 cells. These two events have frequently been associated with tumor invasion and escape from immune surveillance (13, 24-26). The down-regulation of these two antigens, along with poor expression of E-cadherin (see below), might have contributed to the disease progression of this patient from the primary site to the regional multiple lymph nodes in the neck.

The low expression of surface CD44s and E-cadherin by the UP-LN1 cell line may also reflect an invasive and progressive phenotype of this lymph node metastasis from which this cell line was derived. These observations are in sharp contrast to the strong surface expression of CD44s and E-cadherin by cultured epithelial cells immortalized with the SV40 large T oncogene (27) (results not shown). E-cadherin is a calcium-dependent cell-cell adhesion molecule. Based on previous studies on its role in tumor behavior, it has been postulated that a loss of E-cadherin expression or function could facilitate tumor invasion (28-31). The UP-LN1 cell line seems to be an appropriate *in vitro* experimental system for studying the detachment behavior of malignant cells, which may be related to their ability to escape from the primary tumor *in vivo*. We believe that the correlations between the UP-LN1 cell phenotype of loose attachment to the culture vessel substrate, the histopathology of the original lymph node metastatic tumor biopsy revealing solid tumor, loosely individual tumor cells and multicell spheroids consisting of small numbers of tumor cells, and the low expression of E-cadherin by UP-LN1 cells are not all coincidental. A study reported by Zamora *et al.* (32) showed the invasion of endothelial cell monolayers growing on collagen gels by cells from murine mammary tumor spheroids of 25-500 μm in diameter. They found that spheroids could attach to endothelial monolayers for approximately 2 hours, followed by local retraction of the monolayer and migration of tumor cells from the spheroid onto the underlying collagen gel. Some tumor cells were able to invade laterally, moving underneath the endothelial cells, while others penetrated into the collagen gel. Thus, this cell line, which is capable of exhibiting the spheroid type of growth, could be used for further studies to understand the roles of some key tumor-associated adhesion molecules and of the interactions among tumor cells, endothelial cells, stromal fibroblastic cells and matrix, in tumor progression, invasion and in the steps involved in the metastatic cascade.

We recently obtained results to show that UP-LN1 cells expressed and shed abundant FasL molecule (results not shown). It was likely that the tumor also expressed this molecule *in vivo*, providing a means to counter-attack cytotoxic T lymphocytes (CTLs) bearing Fas in the patient (33). In this way, the tumor delivered the death signal to activated CTLs, another mechanism of tumor immune evasion.

The SKY and CGH analyses identified several chromosomal abnormalities and the results obtained from these two approaches adequately corroborated with each other in terms of interpretation. The chromosome content of UP-LN1 cells determined by karyotyping was between 73 and 76 (~ 3n). This hypertriploid chromosome content was also in agreement with the DNA index of 1.7 as determined by the nuclear DNA measurements. Seven different translocations were observed, four of which involved chromosome 19. Whether any of these translocations leads to the creation of oncogenic fusion genes and functional chimeric proteins, as shown in other tumor types (34), remains to be determined. Numerical imbalances were further assessed by comparative genomic hybridization. Our CGH study demonstrated gains of regions from chromosomes 1, 3, 5, 11, 17 and 21, and losses of regions from chromosomes 2, 3, 4, 10, 18 and 21. These abnormalities may have been involved in certain genetic pathways in the development of progressive (invasive/metastatic) cancer. While the significance of all of the abnormalities is not immediately clear, a comprehensive comparison of genetic profiles of lymph node metastasizing and non-metastasizing carcinoma cells for a given type of tumor may uncover specific genetic alterations involved in the invasive and metastatic process.

In summary, we have established a cell line, UP-LN1, from a poorly-differentiated lymph node metastasis of an epithelial tumor of suggested gastrointestinal origin. This cell line exhibits a rather unusual growth pattern because of the presence of loosely attached aggregates (colonies) in addition to typical attached epithelial cells. These features could also be observed at histopathological examination of the biopsy tissue. The down-regulation of surface HLA-class I antigens, CD44s, CD44v5, CD44v6 and E-cadherin molecules in the UP-LN1 cells may all have contributed to the invasive phenotype exhibited clinically. Because of the unusual *in vitro* and *in vivo* characteristics observed, the UP-LN1 cell line should be useful for further studies of tumor invasion and metastatic process.

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