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MORPHOLOGIC, IMMUNOLOGIC, BIOCHEMICAL, AND CYTOGENETIC CHARACTERISTICS OF THE HUMAN GLIOBLASTOMA-DERIVED CELL LINE, SNB-19

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SUMMARY

Human glioma-derived cell cultures and lines have proven to be of significant value in the study of the basic properties that contribute to the highly malignant, invasive and angiogenic phenotype of glioblastoma multiforme tumors. It is frequently difficult to establish lines that retain glial tumor properties in long term culture. The SNB-19 cell line has maintained and exhibited properties of transformation, differentiation, autocrine growth response, and tumorigenesis while remaining in culture for over 13 yr and undergoing over 200 passages. This human line has been utilized in a wide range of studies related to the basic properties of human glioblastoma multiforme. In this report, we summarize the immunologic, biochemical, and cytogenetic properties of this versatile cell line and its utility for additional mechanistic investigation into the pathophysiology of the progression of human malignant gliomas.

Key words: cell line; GFAP; glioblastoma; basic fibroblast growth factor; chromosome.

INTRODUCTION

Human glioma-derived cell cultures and lines have proven to be of significant value in the study of the basic properties that contribute to the highly malignant, invasive, and angiogenic phenotype of glioblastoma multiforme tumors. It is frequently difficult to establish long-term cell lines that retain distinctive glial tumor properties. The SNB-19 cell line has maintained glial cell characteristics over a 13-yr period and is now in its 200th subculture. This cell line exhibits biological characteristics that reflect the intrinsic properties associated with a glioblastoma multiforme *in vivo*. Specifically, SNB-19 cells proliferate rapidly, grow in soft agar, produce a potent angiogenic growth factor (basic fibroblast growth factor, bFGF), and exhibit high levels of protease activity. Even after long-term culture, these cells are capable of producing tumors in the nude mouse that are indistinguishable from the original tumor. This line also responds to differentiating agents and exhibits a highly complex, near-triploid chromosome pattern. This report provides new information and summarizes the work of a number of investigators who have used this cell line for a variety of studies that examine the basic properties attributed to human glioblastoma multiforme.

MATERIALS AND METHODS

The SNB-19 cell line was derived from the surgical resection of a left parieto-occipital glioblastoma multiforme tumor from a 47-yr-old male (12). The resection was performed at the National Institutes of Health in 1980. Tumor tissue was minced into progressively smaller pieces and aspirated through smaller pipettes until tumor cells were evenly dispersed (5). The cell suspension was placed in 25-cm² flasks containing F10 nutrient medium (GIBCO, Grand Island, NY) with 20% calf serum (GIBCO). The flasks were incubated at 37° C without CO_2. Conservative subculturing as the cells approached confluence was performed by gently trypsinizing the cells and washing the detached cells in medium. Initial subculturing was performed at a 1:2 ratio, but routine subculturing is now performed at ratios of up to 1:20. Mycoplasma testing using an enzyme immunoassay kit (Boehringer Mannheim, Indianapolis, IN) has yielded consistently negative results. Cryopreservation of the cells was performed by trypsinizing logarithmically growing cells in serum, media, and dimethyl sulfoxide (Sigma, St. Louis, MO) in a 2:2:1 ratio. The cells remain viable for over a year using this technique and are thawed by rapid warming to 37° C, washing 3 times in serum-supplemented growth medium and plating them on polystyrene flasks (PGC Scientific, Gaithersburg, MD).

Kinetic parameters. Cells were grown in sealed polystyrene flasks with F-10 medium, 1% penicillin/streptomycin (PCN/STR, GIBCO), and 15% calf serum in a CO_2-free incubator maintained at 37° C. Tumor cells have also been maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO) with 10% fetal bovine serum (FBS, GIBCO), 0.6% L-glutamine, 0.1% sodium pyruvate, 1% PCN/STR at 37° C in a 5% CO_2 incubator. The cells were allowed to plate for 48 to 72 h and counted after 5 or 6 days of growth. Alternatively, cells have been plated in a serum-supplemented medium and converted to a chemically defined medium (CDM) (20) consisting of a 1:1 mixture of Ham's F12 (GIBCO) and DMEM, 50 n*M* hydrocortisone (Sigma), and 5 μg/ml insulin (Sigma), prostaglandin F2α (500 ng/ml) (Sigma), transferrin (50 μg/ml) (Sigma) and 100 μ*M* putrescine (Sigma). Cell counts were performed after 5 to 8 days of growth.

Morphologic characterization and response to differentiating agents. Cells were grown on glass slides and fixed in ethanol and acetone. Hematoxylin

and eosin or Giemsa stains were used to examine the cells microscopically. Differentiation was induced by incubating the cells in 1 m*M* dibutyryl cAMP (Sigma) and 1 m*M* 3-isobutyl-1-methylxanthine (Sigma), a phosphodiesterase inhibitor (Fig. 1).

Cytogenetic analysis. Cells at Passage 163 were arrested in metaphase by treatment for 4 h in Colcemid (0.1 μg/ml, Irvine Scientific, Santa Ana, CA). Ethidium bromide (10 μg/ml, Fisher Scientific, Pittsburgh, PA) was added to the culture 3 h before termination of Colcemid action to retard chromosome condensation. After brief trypsinization to release the cells from the flask, the cells were swollen in hypotonic 0.075 *M* KCl for 24 min at 37° C, and fixed in 3:1 methanol:acetic acid. Slides were prepared, baked overnight, and banded by a modified Klinger trypsin-Giemsa method (18). Cytogenetic analysis of 20 metaphase cells was carried out, and karyotypes were expressed in accordance with the International System for Cytogenetic Nomenclature (15).

Immunologic characterization. Cells were grown on 18-mm-square cover glasses as described in Kinetic Parameters. The slides were rinsed twice with Hanks' balanced salt solution (HBSS) without calcium and magnesium (CMF-HBSS, GIBCO), and fixed for 10 min at 37° C in 2% paraformaldehyde in the same buffer. The cells were washed 3 times at room temperature with gentle agitation in CMF-HBSS. Cells were dehydrated in acetone (−60° C) for 1 min, washed as above, blocked for 30 min at room temperature in CMF-HBSS containing 0.5% bovine serum albumin (BSA), and washed again. Test cells were incubated for 1 h at room temperature in CMF-HBSS containing 0.5% BSA, rabbit antiglial fibrillary acidic protein (GFAP) diluted 1:25 (Sigma) and 0.5 U rhodamine phalloidin/cover glass (Molecular Probes, Eugene, OR) for GFAP and actin visualization, respectively. After washing the cells as above, they were incubated for 1 h at room temperature in CMF-HBSS containing 0.5% BSA and a 1:50 dilution of biotinylated goat anti-rabbit IgG (Sigma), washed, incubated in CMF-HBSS containing 0.5% BSA and 5 μg/ml Cy 5.18-labeled streptavidin (a gift from Dr. A. Waggoner, Carnegie Mellon University, Pittsburgh, PA), and washed a final time.

Intracellular GFAP was also identified using Western immunoblots in a method described by Welch and colleagues (33). Briefly, logarithmically growing cells were washed in HBSS or DMEM and iced HBSS or DMEM with the addition of leupeptin (10 μg/ml, Sigma) and phenylmethylsulfonyl fluoride (PMSF, 35 μg/ml, Sigma). The extracted cells were placed in an iced buffer consisting of 10 m*M* tris-HCl, pH 7.0, 2 *M* NaCl, 10 μg/ml leupeptin, 35 μg/ml PMSF, and 0.1% (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) (CHAPS, Sigma). They were ultrasonically disrupted and centrifuged at 14 000 ×*g* at 4° C for 15 min to remove membrane proteins. The soluble protein concentration was determined by the Lowry protein assay (19). Protein samples (10 to 40 μg) were diluted in sample buffer (0.0625 *M* tris, 10% glycerol, 5% beta mercapto-ethanol, 3% sodium dodecylsulfate, Sigma), 1% bromophenol blue/xylene cyanate (Sigma) and B-mercaptoethanol (Sigma), and run on a 4% stacking/12 or 15% separating sodium dodecyl sulfate (SDS) polyacrylamide gel (26) at 50 V for 30 min and 150 V for 1 h with molecular weight markers. The separated proteins were transferred onto a positively charged nylon membrane (Immune-Lite, Bio-Rad, Melville, New York) in a non-reducing buffer (25 m*M* tris, 192 m*M* glycine, and 20% methanol) at 450 mA constant current. The membrane was blocked with 5% non-fat dry milk in 20 m*M* tris, 500 m*M* NaCl, pH 7.5 for 30 min at 37° C and incubated overnight at 4° C in 50 m*M* tris-HCl, pH 7.8, 1% BSA, 1 m*M* $MgCl_2$, 0.02% sodium azide with mouse monoclonal antibody directed against GFAP (diluted 1:20, Sigma). Unbound primary antibody was removed by washing with Buffer W (1.5 m*M* KH_2PO_4, 8.0 m*M* Na_2PO_4, 140 m*M* NaCl, 3.0 m*M* KCl, 0.05% Tween 20, pH 7.4). Incubations with biotinylated goat anti-mouse antibody (Sigma, 1:50) and streptavidin-alkaline phosphatase conjugate (Amersham 1:3000 dilution) were performed as described above. The membrane was then washed 3 times in Buffer WS (Buffer W with 0.9 *M* NaCl) and twice in 20 m*M* tris, pH 7.5, 500 m*M* NaCl to reduce background. The membrane was placed between two acetate sheets with 0.5 ml chemiluminescent substrate (Boehringer Mannheim) and allowed to incubate at room temperature for 4 to 10 h. The membrane was then exposed to x-ray film (Kodak X-AR, Rochester, NY, or Fuji RX, Fisher) for 5 to 30 s and the film was developed. Membrane re-probing can be performed by washing in the last buffer and renewing the chemiluminescent substrate.

Intracellular basic fibroblast growth factor determinations were performed using the Western immunoblot technique described above. Human recombinant bFGF (Upstate Biologicals Inc., Lake Placid, NY) was used in each gel to serve as an internal control, and mouse monoclonal anti-bFGF (Upstate Biologicals Inc., 1 μg/ml) was used as the primary antibody. A scanning densitometer (PDI Molecular Dynamics or Hoefer GS300 with GS370 software) was used to determine bFGF concentrations and our level of sensitivity was 1 to 2 ng.

Invasiveness Characterization.

Identification and quantitation of cell-associated protease activity. Tumor cells from SNB-19 and two other human glioma-derived cell lines were grown to near confluence in F10 medium as described above. The cells were scraped free from the 75-cm^2 polystyrene flasks in 1 ml 0.25 *M* sucrose in 5 m*M* *N*-[2-hydroxyethyl] piperazine-*N'*-[2-ethansulfonic acid] (HEPES buffer, Sigma), 0.01% Triton X-100 (vol/vol), pH 7.3, buffer. The cells were homogenized on ice using 20 strokes, and a protein assay was performed (bicinchoninic acid protein assay kit, Sigma) (28).

Trypsin-like protease activity was determined by assessing the rate of cleavage of the peptide substrate Boc-Phe-Ser-Arg-7-amido-4-methylcoumarin (32). Specifically, a 10-μ*M* concentration of peptide in 0.1% Triton X-100, 0.1 *M* tris buffer, pH 8.9, was made (32). A 50-μl sample of cellular protein was added in triplicate trials to the peptide and buffer and periodic fluorescent intensity measurements (Microfluor fluorescence plate reader, Dynatech, Chantilly, VA) were performed at 37° C after incubation at 0, 5, 10, and 20 min. Results were expressed in picomoles 7-amido-4-methylcoumarin (AMC) released per minute per milligram cellular protein by comparison of the fluorescence intensity of the samples to that of a 1 μ*M* AMC standard.

Chymotrypsin-like protease activity was determined using the peptide cleavage of Suc-Ala-Ala-Phe-AMC in the fashion described above.

SDS-PAGE zymography to visualize protease activity through the digestion of gelatin (Sigma) (14). Approximately 30 μg of protein sample was placed in a 4% stacking (0.125 *M* tris-HCl, pH 6.8, 0.1% SDS) and 7% separating (0.375 *M* tris-HCl, pH 6.8, 0.1% SDS, 1 mg/ml gelatin) polyacrylamide slab gel. The proteins were run for 45 min at 45 mA in a 4° C coldbox to prevent non-specific gelatin digestion. The samples were not boiled before electrophoresis so as to maintain enzymatic activity. The gel was washed for 30 min at room temperature in 50 m*M* tris-HCl, pH 7.5, 5 m*M* $CaCl_2$, 1 μ*M* $ZnCl_2$, 2.5% Triton X-100, and incubated at 37° C overnight by shaking in a similar buffer with 1% Triton X-100. The gel was stained in a 0.1% Coomassie blue solution.

Plasminogen activator (PA) activities (urokinase-type and tissue-type) had been performed on Triton X-100 extracted cells using a ^{125}iodine-labeled fibrin plate assay (12) with comparisons against standard human urokinase (Leo Pharmaceutical Products, Helsingborg, Sweden). PA type was determined by performing the assays in the presence or absence of fibrin.

Growth in vivo. *In vivo* growth of these cells has been performed by explanting cells in the subcutaneous areas of nude mice (12) as follows: SNB-19 cells were trypsinized, re-suspended in medium, centrifuged, and re-suspended at 1 × 10^8 cells/ml in 0.9% NaCl. The subcutaneous areas of mice were inoculated with 10^7 cells, and tumor growth was determined by measurement. The tumors were examined histopathologically after the animals were killed.

Growth in soft agar. Growth in soft agar was determined by suspending 9 × 10^4 cells from four glioma cell lines/60-mm dish in 0.5% (wt/vol) Bactoagar (Difco Laboratories, Detroit, MI) in growth medium. This suspension was overlaid on a hardened 1% (wt/vol) agar-growth medium base. These cells were fed biweekly, and colonies of 50 or more cells were counted at 1 mo. (12).

Growth factor analysis. Response of SNB-19 cells to exogenous bFGF stimulation was performed in F10 medium, 1% PCN/STR and 15% calf serum in a CO_2-free incubator maintained at 37° C using human recombinant bFGF. Alternatively, a similar chemically defined medium (21) consisting of a 1:1 mixture of Ham's F12 (GIBCO) and DMEM, 30 n*M* selenium (Sigma), transferrin (50 μg/ml), 10 n*M* hydrocortisone, and 5 μg/ml insulin was used in the following fashion: cells were plated in serum-supplemented medium and maintained in a serum-free medium (DMEM, 0.6% L-glutamine, 1% PCN/STR) for 20 h before conversion to this chemically defined medium. The cells were then exposed to increasing amounts of bovine bFGF (22) and counted after 6 days of growth.

Results

Doubling time. The growth of SNB-19 cells was initially observed in Ham's F10 medium with 15% calf serum. The cells were allowed

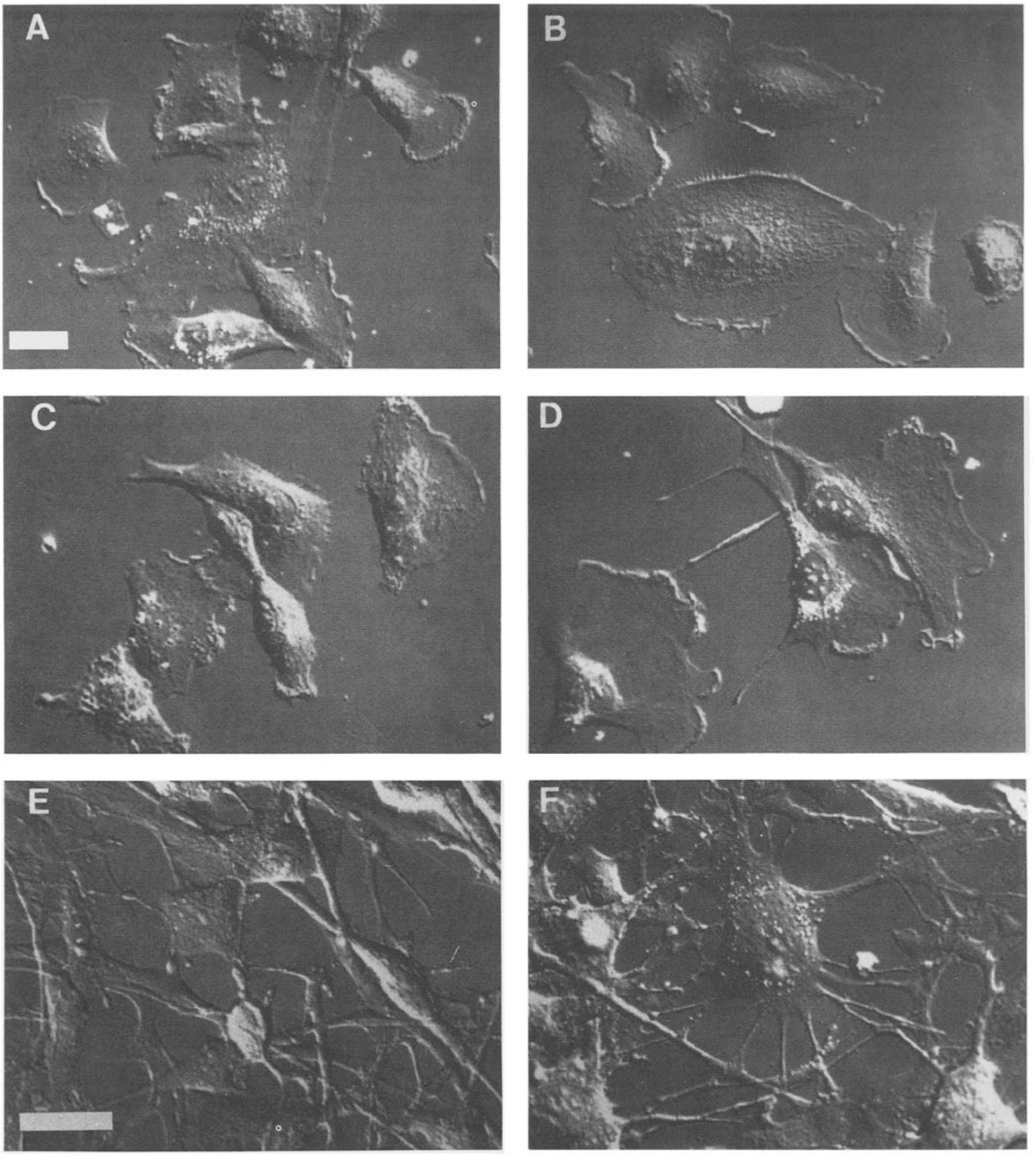

FIG. 1. The morphology of SNB-19 cells cultured in various growth media and in the presence of a differentiating agent. SNB-19 cells were plated onto glass cover slips at a density of 3500 cells/cm^2 and incubated at 37° C in a 5% CO_2 atmosphere (*A–C,E,* and *F*) or at 37° C in sealed petri dishes (*D*) for 24 h. We observed the cells with video-enhanced differential interference contrast microscopy (1) using either a 40× Plan-Neofluar (1.30 N.A.) or a 63× Plan-Neofluar (1.25 N.A.) objective. The various growth media used were as follows: *A*, DMEM with 10% FBS; *B*, CDM, no serum; *C*, DMEM with 10% calf serum; *D*, F10 with 15% calf serum. To induce differentiation (*E,F*), cells were incubated for an additional 24 h in DMEM containing 1 m*M* dibutyryl cAMP and 1 m*M* 3-isobutyl-1-methylxanthine. Undifferentiated SNB-19 cells in the various types of growth media maintained a similar morphology. Many cells exhibited the morphology of highly motile cells (i.e. an extensive lamellipodia with constant ruffling activity at the leading edge, a nucleus located at the rear of the lamellipodia, and a contracting tail). Differentiation induced the appearance of processes (*E*) that seemed to be extensive in some cells (*F*). *Scale bar* in *A* is 30 μm and pertains to panels *A–D*. *Scale bar* in *E* is also 30 μm and pertains to panels *E* and *F*.

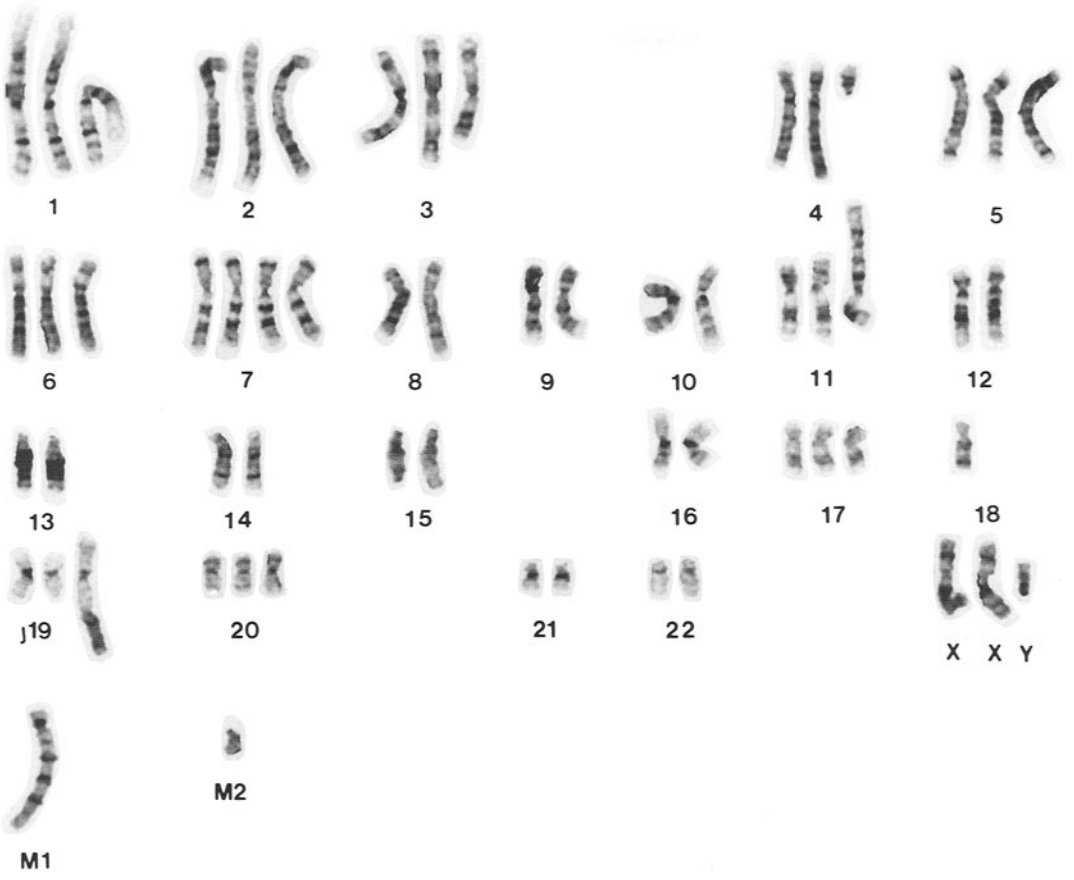

FIG. 2. G-banded karyotype of a representative SNB-19 cell at Passage 163.

to plate for 48 h and counted at 6 days. The cellular doubling time was 21 h at 37° C. The doubling time of cells maintained in DMEM at 37° C with 10% FBS and 5% CO_2, was 36 h. Growth in serum-free CDM was possible with SNB-19 cells, but the cells grew too slowly to accurately determine doubling times.

Morphology and response to differentiating agents. SNB-19 cells maintained a similar morphologic appearance in F10, DMEM, or CDM (Fig. 1). The cells were typically small, relatively uniform and exhibited few processes. Morphological differentiation, as evidenced by the extension of dendritic processes, occurred with the addition of 1 m*M* dibutyryl cAMP and 1 m*M* 3-isobutyl-1-methylxanthine (Fig. 1).

Cytogenetic analysis. Classical cytogenetic analysis of 20 trypsin-Giemsa banded metaphase cells from Passage 163 revealed a highly complex, abnormal, near-triploid chromosome pattern, including both numerical and structural abnormalities (Fig. 2). The consensus karyotype, listing those abnormalities present in 50% or more of cells, was:

53–69<3n>,XXY,der(3)t(3;13)(q12↕2),del(4)(q12),
−6,+7,−8,−9,−10,der(11)t(3;11)(q11↑5),−12,−13,
−14,del(15)(q15q21), −16,−18,−18,der(19)t(1;14;19)
(1pter→1p34::19p13→19q13::14q32→14q12),
−21,−22,+mar 1.

Immunologic analysis. SNB-19 cells typically demonstrated moderate, uniform immunoreactivity for GFAP and actin with no tendency for the two proteins to colocalize (Fig. 3). GFAP immunoreactivity was also identified on Western immunoblots (data not shown).

Invasiveness assays: Protease activity. Trypsin-like protease activity was found to be high in SNB-19 cells (497 pmol·min^{-1}·mg^{-1} cellular protein). Chymotrypsin-like activity of SNB-19 cells was 11.1 pmol·min·mg cellular protein. PA activity was the highest in SNB-19 cells of four glioma lines tested (3.20 vs.2.00, 0.05, and 0.17 m Plough U/ug cellular protein) (12).

Growth in soft agar and nude mice. Clonogenicity in soft agar was determined by the methods outlined by Dexter and colleagues (8) and SNB-19 cells were found to be 41.5% clonogenic, whereas the other three glioma cell lines tested were less than 10% clonogenic (12). SNB-19 cells were the most tumorigenic of four glioma lines tested in nude mice. Tumors appeared 30 to 35 days after injection (12) and the mice were killed at 97 days postinjection due to excessive tumor burden (average tumor weight 6.8 g).

Response of cells to bFGF stimulation. SNB-19 cells growing in CDM exhibited a twofold increase in cell density in response to the addition of bovine bFGF (21) (Fig. 4). This growth response was saturable and dose-dependent. Cells growing in F10 medium also responded to the exogenous addition of human recombinant bFGF from 1 to 5 ng/ml, but this growth response was not as marked as that seen in CDM (data not shown). Interestingly, the maximal stimulation occurred at 5 ng/ml and growth reduction occurred at 10 ng/ml.

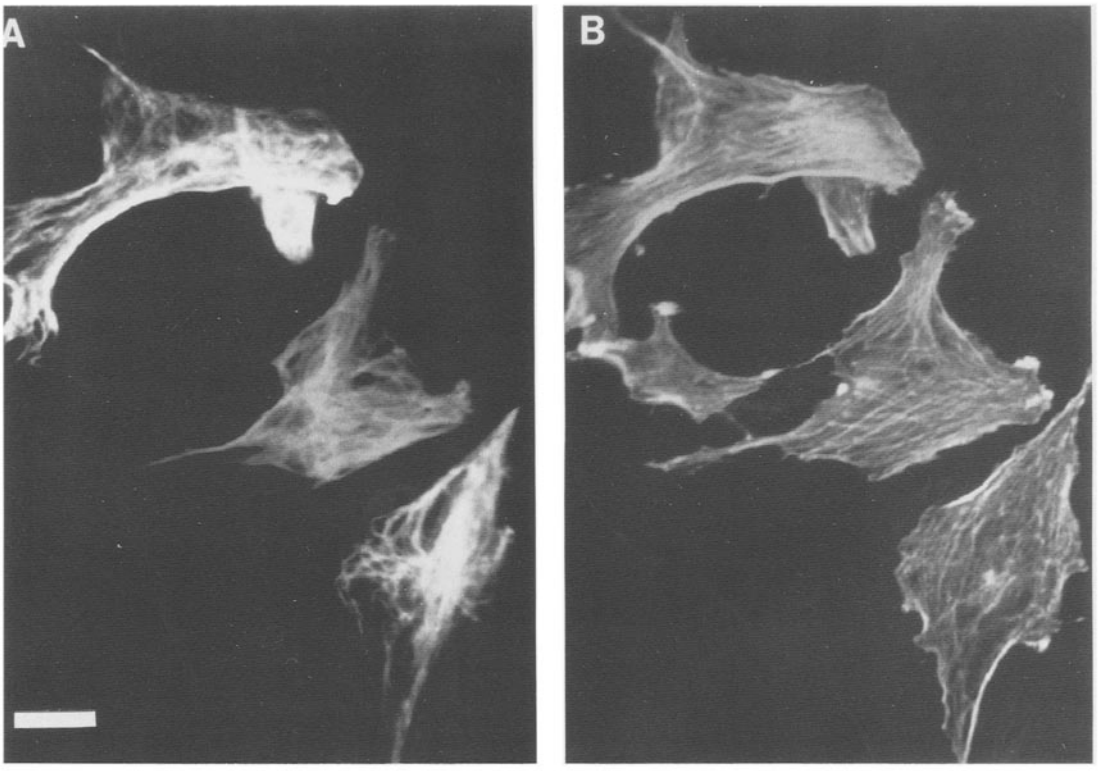

FIG. 3. SNB-19 cells at Passage 193 contain a network of GFAP and actin fibers. Cells were grown on cover glasses, rinsed with CMF-HBSS, and fixed in 2% paraformaldehyde. Cells were washed, dehydrated in acetone (−60° C), washed again, and blocked in CMF-HBSS containing 0.5% BSA. Another wash was performed and the cells were incubated with rabbit anti-GFAP (1:25) and 0.5 U rhodamine phalloidin/cover glass (Molecular Probes) for GFAP and actin visualization, respectively. After washing the cells, they were incubated with biotinylated goat anti-rabbit IgG (1:50), washed, incubated with 5 μg/ml Cy 5.18-labeled streptavidin, and washed a final time. A Zeiss 40×, 1.3 NA Plan-Neofluar objective and a cooled CCD camera was used to record the fluorescence images. *A*, immunolocalization of GFAP fibers. Fibers extended throughout the cytoplasm of these cells. Control experiments using no anti-GFAP antibodies showed no fluorescent signal above the cellular autofluorescence signal. *B*, localization of actin fibers in the same cells as shown in *A* using rhodamine phalloidin. Fine stress fibers containing actin were dispersed throughout the cytoplasm of SNB-19 cells just as GFAP fibers were, but there was no tendency for the two proteins to colocalize. *Bar* = 25 μm.

Cellular production of bFGF. SNB-19 cells express bFGF protein and mRNA (20). Western immunoblot analysis has shown intracellular bFGF concentrations to be consistently greater than 1.0 ng/μg protein in logarithmically growing cells (Fig. 5).

DISCUSSION

We have found the SNB-19 human glioblastoma-derived cell line to be a useful model for examining the properties of cells derived from malignant glial neoplasms. Specifically, the cells grow well in different types of media, have similar doubling times in different types of media, and do not require a CO_2 environment. The cells exhibit a transformed phenotype as demonstrated by their ability to survive *in vitro* for more than 200 passages, grow in serum-free medium, and form subcutaneous tumors in immunocompromised mice. The ability of the cells to grow in soft agar without adhering to surfaces provides further evidence of their transformed phenotype.

Cytogenetic analysis of SNB-19 glioblastoma cells arrested in metaphase demonstrated relative cellular heterogeneity as few cells are identical based on karyotype. Consistent chromosomal abnormalities, the most notable of which are a hyperdiploid karyotype, a gain of chromosome 7, and a loss of chromosome 10, are also noted upon review of the cytogenetic structures. These abnormalities have been noted in other glioma-derived cell lines (3,6,16). The chromosomal analysis demonstrates that the SNB-19 cell line exhibits genetic alterations that are consistent with those observed in glial tumors and glioma cell lines.

Glial fibrillary acidic protein is an intermediate filament found in astrocytes (9). The presence of this protein confirms the glial nature of the cells and suggests that the cells were relatively well differentiated (24). Glial cells grown in long-term culture frequently lose their cellular GFAP immunoreactivity (4,6,10,24) suggesting that they have become less well differentiated. Despite the transformed and tumorigenic nature of SNB-19 cells, they continued to express immunoreactivity to anti-GFAP antibodies as assessed by Western immunoblot or immunochemical techniques (Fig. 3). Interestingly, SNB-19 cells also developed dendritic processes when stimulated with the differentiating agent, cAMP. These findings reinforce the neoplastic nature of the cells and suggest that the cells can exhibit the morphologic changes associated with differentiation (7).

Biochemical characterization of invasiveness includes determination of the expression of cellular proteases such as PA. PA has

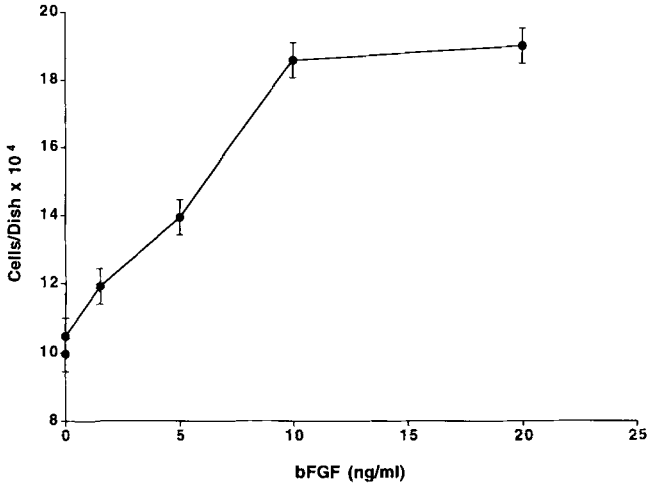

FIG. 4. Growth response of SNB-19 cells as a function of bovine pituitary bFGF concentration. Concentration range was 0.1 to 20 ng/ml. Cells were plated and maintained in a 1:1 mixture of Ham's F12 (GIBCO) and DMEM, supplemented with 20% (vol/vol) heat inactivated (56° C, 30 min) FBS (Hyclone Laboratories, Logan, UT) with 5% CO_2 at 37° C. Six days after conversion to a 1:1 mixture of Ham's F12 and DMEM, 30 n*M* selenium, transferrin (50 μg/ml), 10 n*M* hydrocortisone, and 5 μg/ml insulin and growth factor addition, cells were trypsinized and counted. Three dishes were used per treatment. Data are expressed as the average number of cells per dish ± SEM, (*bars*). [Adapted from Morrison et al., with permission (21)].

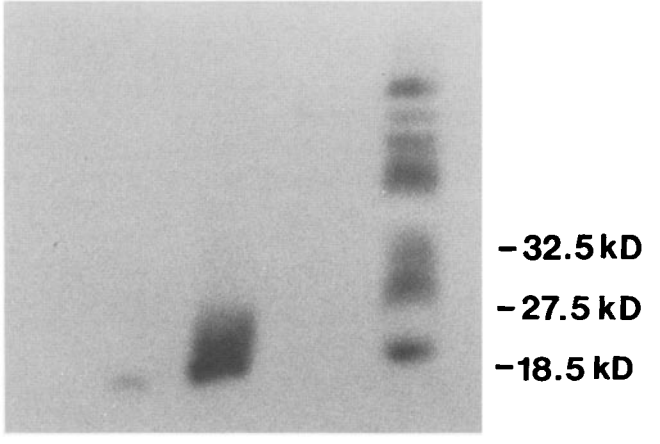

FIG. 5. Western immunoblot analysis demonstrating bFGF concentrations to be greater than 1.0 ng/μg protein. Logarithmically growing cells were washed in the presence of anti-proteases, and placed in an iced buffer containing CHAPS. Cells were ultrasonically disrupted, centrifuged, and the soluble protein concentration was determined. Samples were diluted in a reducing buffer and run on an SDS-polyacrylamide gel with commercially purchased human recombinant bFGF (Upstate Biologicals Inc.) as an internal control. The separated proteins were transferred onto a nylon membrane in a non-reducing buffer and the membrane was blocked. A mouse monoclonal antibody directed against bFGF (Upstate Biologicals Inc.) was incubated with the membrane, and unbound first anti-body was removed by washing. Incubations with biotinylated goat anti-mouse antibody and streptavidin-alkaline phosphatase conjugate were performed. The membrane was washed and incubated with a chemiluminescent substrate. The membrane was then exposed to x-ray film and the film was developed. A scanning densitometer was used to determine sample intracellular bFGF protein concentrations.

been associated with the expression of the malignant phenotype (25), and SNB-19 cells produce elevated levels of PA. This protease functions through the proteolytic conversion of plasminogen to plasmin (31), presumably facilitating tumor invasion directly via plasmin-mediated destruction of matrix glycoproteins and indirectly by stimulating cell growth and activation of cytokines [for review, *see* (30)]. Human malignant astrocytoma-derived tumor cells produce increased amounts of PA (23) and PA production is stimulated by bFGF in non-astrocytic cell lines (23).

Proteases other than PA may also be involved in tumor invasion. Recently, non-granular cytosolic proteases have been identified in rat natural-killer cells (11). These proteases are constituents of a protein complex that may influence cell division and homeostasis (27), cell mediated cytotoxicity (11), and embryonic cell migration (17). SNB-19 cells produced high levels of trypsin-like activity compared to other glioblastoma multiforme-derived cells and chymotrypsin-like activity similar to other cells (R. H. Goldfarb, personal communication, 1994). The function of these proteases in malignant astrocytoma-derived cells remains unclear, but they may also participate in tumor invasion.

Arai and colleagues (2) identified an anaplastic astrocytoma-derived cell line that was GFAP positive and secreted an uncharacteristic growth-promoting factor into the culture medium. Sporn and Roberts (29) have forwarded the hypothesis that cancer cells exhibit a positive autocrine-type stimulation by producing both a growth factor and the receptor for this factor. One possible candidate for an autocrine growth factor in gliomas is bFGF. SNB-19 cells produce bFGF as demonstrated by both slot-blot (20) and Western immunoblot analyses. These cells also expressed high affinity bFGF receptors (21) and responded to the exogenous addition of bFGF with increased cell growth in serum-supplemented or serum-free medium. Furthermore, these cells depended on bFGF stimulation to maintain their rapid growth. This is evidenced by the substantial growth inhibition that occurs when bFGF-specific antisense oligonucleotides were added to the extracellular growth medium (20). As such, these cells fulfilled the criteria for autocrine stimulation by bFGF (13).

CONCLUSION

This cell line has retained glial properties as demonstrated by its continued expression of GFAP immunoreactivity despite its *in vitro* growth for over a decade. SNB-19 cells also exhibited features consistent with malignant transformation, including growth in soft agar and nude mice, differentiation with the addition of cAMP in serum-deprived medium, and autocrine growth responses. The cell line had a highly complex, near-triploid chromosome pattern and exhibited the most common glial-derived cellular chromosomal abnormalities, including gain of chromosome 7 and loss of chromosome 10. These cells grew in different types of media, survived in reduced serum concentrations, and produced proteases. As such, this cell line exhibited properties of transformation, differentiation, invasiveness, autocrine growth response, and tumorigenesis. This summary of the morphologic, immunologic, biochemical, and cytogenetic properties of this cell line may provide a useful framework of reference for researchers interested in examining the molecular dynamics of invasive motility, signal transduction as it relates to differentiation, and other specific properties of this malignant glial-derived cell line.

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