A COMMON CELL-TYPE SPECIFIC SURFACE ANTIGEN IN CULTURED HUMAN GLIAL CELLS AND FIBROBLASTS: LOSS IN MALIGNANT CELLS

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Previous studies have shown that normal chicken and human fibroblasts contain a major cell-type specific surface antigen (fibroblast surface antigen, SFA). It is a high molecular weight glycoprotein (1, 2) that is lost after transformation by RNA or DNA viruses (3, 4). Virus-transformed fibroblasts lack surface SFA but produce it to the culture medium. In normal fibroblasts SFA (for summary see reference 5) is mainly located in fibrillar structures of the cell surface and in cellular processes (6). Considerable quantities of the antigen are also shed from normal cultured fibroblasts and it is also present in circulation (plasma and serum), presumably as a result of shedding in vivo. Serum SFA shows affinity to fibrinogen and fibrin (7) and is identical with what has been known as the "cold insoluble globulin" of human plasma (8). SFA was not found in several other cell types including parenchymal cells of various organs, lymphocytes, erythrocytes, amnion, lymphoma, and carcinoma cells (1, 2, 9) but has been constantly detected in fibroblasts in vivo and in vitro.

The close functional relationship between fibroblasts and glial cells has led us to study the latter cell type for the presence of SFA. Using several characterized normal and malignant human glial cell lines we now show that cultured glial cells have SFA immunologically indistinguishable from that of fibroblasts and that on cell surface it is also distributed to fibrillar processes. The data also show that malignant glioma lines established from human tumor patients produce SFA but retain little or none on cell surface.

Materials and Methods

Cells. All cell lines were grown at 37°C in Eagle's basal medium plus 10% fetal calf serum and were regularly subcultured twice a week using a solution of 0.25% trypsin and 0.02% EDTA in phosphate-buffered saline (PBS) to disperse the cells.

The normal glial cell lines have been grown from explant cultures of non-neoplastic adult human tissue obtained from neurosurgical operations as described by Pontén and Macintyre (10).

* This work was supported by grants from the Cilag Chemie Foundation, the Finnish Cancer Foundation, the Finnish Medical Research Council, the National Cancer Institute (IR01 CA17373-01), and the Swedish Cancer Society.

Abbreviations used in this paper: anti-SF-FIBR, antibodies produced against fibroblast surface SFA; anti-SF-NHP, antibodies produced against SFA purified from normal human plasma; PBS, phosphate-buffered saline; RIA, radioimmunoassay; SFA, fibroblast surface antigen.
The three lines, MIRI (kindly provided by Dr. T. Wahlström, Third Department of Pathology, University of Helsinki, Helsinki, Finland), U-787 CG, and U-813 CG represent diploid astroglial cells, have an astrocyte-like morphology, a finite life-span (about 25 cell generations), and a low terminal saturation density (Table I and references 11, 12). All experiments have been performed on cells in the phase of rapid proliferation [phase II according to Hayflick (13)].

The origin of the glioma lines has been described (14). Some of their properties are given in Table I. Because of the absence of spontaneous infinite growth transformation among normal glial cells, the infinite life-span of the glioma cells in culture is a reliable criterion on their neoplastic origin. Production of the neuroectoderm-specific protein S-100 (16) by some representative lines of the panels of normal (K. Haglid, and B. Westermark, unpublished data) and malignant (17) glial lines is in agreement with their glial origin. The origin of the normal (WI-38) and SV40-transformed (WI-38/VA-13) human fibroblasts was reported earlier (4).

**Anti-SF Antibodies and Their Specificity.** We have used two types of anti-SF antibodies in these studies (a) antibodies produced against fibroblast surface SFA (anti-SF-FIBR) and (b) antibodies produced against SFA purified from normal human plasma (anti-SF-NHP). For preparation of anti-SF-FIBR, digests of extensively washed live fibroblast cultures (MRC-5 line) were obtained using brief treatment with matrix-bound papain. The digests were clarified by ultracentrifugation, dialyzed, and used to immunize sheep. After absorption with calf serum (a component of the fibroblast growth medium) and with insolubilized iodoacetamide-inactivated papain, the antisera have given a single precipitation line against the immunogen, concentrated supernates of spent medium of fibroblast cultures, and against human serum and plasma (2). It is known that the monospecificity of such seemingly crude antisera is most probably due to two things: SFA in cultured fibroblasts is a major surface component and is highly susceptible to release from the cell surface by proteolysis, e.g. by papain, trypsin, and plasmin (references 2 and 3, and footnote 2). To ensure specificity for SFA at the level of immunofluorescence and radioimmunoassay the antisera have been absorbed extensively with purified human blood leukocytes (gift from Dr. G. Myllylä, The Finnish Red Cross Blood Transfusion Center, Helsinki, Finland) using 100 µl of packed leukocytes/ml of serum. For the routine immunofluorescence the IgG fraction of such absorbed antisera was used as such. To check the specificity of the immunofluorescence tests anti-SF antibodies have been further purified by affinity chromatography. Anti-SF antibodies were bound to an immunoadsorbent prepared by conjugating human serum protein (including serum SFA) to agarose beads (Sepharose 4B, Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). The beads were washed with PBS, and the bound antibodies were eluted with 8 M urea (18). The eluate was dialyzed against distilled water and lyophilized. For preparation of anti-SF-NHP, human plasma SFA was purified by cryoprecipitation, DEAE-cellulose chromatography, and isoelectric focusing (7) and used to immunize rabbits.

The two types of antibody preparations, anti-SF-FIBR and anti-SF-NHP, reacted identically in immunodiffusion tests and in immunofluorescence against cells or materials derived from them. The immunofluorescence reactions were blocked totally by either highly purified plasma SFA, papain digests of fibroblast cultures, or with normal human serum but not by bovine or chicken serum.

**Immunofluorescence.** Confluent cultures were seeded in a 1:4 ratio, grown on cover slips, and used at 1-day old. For total immunofluorescence the cover slip cultures were fixed with 3.5% HCHO and acetone and then stained. For surface immunofluorescence the cultures were rinsed with ice-cold PBS, stained at ±0°C and then fixed (HCHO also at ±0°C). With anti-SF-FIBR we used direct and with anti-SF-NHP indirect immunofluorescence as described in more detail elsewhere (4).

**Papain Digests of Glial Cells and Antiglial Papain Digest Antisera.** These were prepared using the U-813 CG cell line and the procedures described above and those described previously for human fibroblast SFA.

**Solubilization of Cellular SFA.** Cellular SFA was solubilized in antigenically active form using a solution containing 8 M urea, 1% Triton X-100, 0.02% NaN₃, and 1 mM phenylmethylsulfonylfluoride (3).

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Table I
The Glia-Glioma System

| Cell line       | Morphology       | Population doubling time in 10% calf serum | Terminal saturation density (× 10^3 cells/cm²) in 10% calf serum |
|-----------------|------------------|------------------------------------------|---------------------------------------------------------------|
| Normal astroglia|                  |                                          |                                                               |
| U-787 CG        | Astrocyte like   | 24–30                                    | 50–90                                                         |
| U-813 CG        |                  |                                          |                                                               |
| MIRI            |                  |                                          |                                                               |
| Malignant astrocytomas |       |                                          |                                                               |
| U-105 MG        | Astrocytoid      | 49                                       | 480                                                          |
| U-118 MG        | Bipolar, spindle | 33                                       | 220                                                          |
| U-251 MG        | Bipolar, spindle | 35                                       | 550                                                          |
| U-343 MG        | Polygonal, spindle | 31                                     | 360                                                          |
| U-343 MG-a*     | Polygonal, spindle | 31                                     | 360                                                          |

References 12, 14, and 15

* A subline of U-343 MG isolated by explantation on a feeder layer of normal glia.

Radioimmunoassay (RIA). A double antibody RIA (18) using 125I-SFA purified from serum (7) has been used. The reaction mixture in the assay consisted of 900 μl of PBS plus 0.2% bovine serum albumin, 100 μl of the sample (or standard) diluted in urea-Triton X-100, 100 μl of 125I-SFA (19) diluted in PBS plus 0.2% bovine serum albumin, and 100 μl of a suitable dilution of anti-SFA.

Results

Glial Cells Contain SFA. Papain-released material and urea-Triton X-100 extracts from human glial cell cultures contained an antigen indistinguishable from the fibroblast SFA in immunodiffusion (Fig. 1). Antiserum produced by immunizing with papain digest from glial cell cultures showed only antibodies which reacted with fibroblast extracts, normal human serum, and SFA purified from human plasma.

Localization of SFA in Normal Glial Cells. In cultures of normal human glial cells, fixed with formaldehyde and acetone, all cells stained strongly positive in immunofluorescence with anti-SFA antibodies. Two types of fluorescence were seen: (a) a fibrillar type of fluorescence that often had a radial pattern (Fig. 2). It extended to the cellular processes, was present also on the growth substrate (the cover slip), and on the extracellular space outside the cell body. The radially orientated "SFA striae" were absent on the blunt side of the cell. (b) Strong "microgranular" SFA fluorescence that is predominantly located in the perinuclear area was seen intracellularly. When glial cells were stained for surface SFA by anti-SFA-antibodies at ±0°C and then fixed only the fibrillar type of fluorescence was seen (Fig. 3).

Localization of SFA in Glioma Cells. In three glioma lines (U-118 MG, U-251 MG, and U-343 MG-a) no or very little SFA was detected in the great majority (>95%) of the cells (like the three negative or weakly positive cells shown in Fig. 4). In line U-343 MG, faint striated fluorescence was seen in some
cells and line U-105 MG was clearly positive, showing both intracellular granular fluorescence and SFA-containing fibrillar striae (Fig. 5). However, even in these cells the intensity of SFA fluorescence was less than that seen in normal glial cells.

A small fraction of cells in several of the cultured glioma lines were intensively positive for SFA. These cells showed "microgranular" cytoplasmic fluores-
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Fig. 3. Surface SFA in normal glial cell (line U-813 CG). Live cell cultures were stained by immunofluorescence in the cold and were then fixed. Note SFA striae in cell periphery. Some nonspecific autofluorescence may be seen in the nucleus. × 750.

cence and in many cases two nuclei (Figs. 4 and 6) suggesting that they were in the telophase stage of the cell cycle.

When cultures of glioma cells were stained by immunofluorescence for surface SFA, lines U-118 MG, U-251 MG, and U-343 MG-a were negative. Weak irregular SFA-containing striae were seen in cells of the U-105 MG line and very faintly in some cells of the U-343 MG line.

Quantitation of SFA in Cells and Growth Media. The concentration of SFA in the cells and the medium 2 days after subculture is given in Table II. The results were essentially similar on day 1 and day 3. The concentration of SFA in normal glial cells exceeds that in normal fibroblasts and is much higher than that in malignant glial cells. In the latter group the concentration was highest in cells of line U-105 MG (Table II), in agreement with the results of immunofluorescence experiments. Both normal and malignant glial cells produce SFA. When plotted against the amount of cell-associated protein present at the time of harvesting the total amount (cells plus medium) present in the malignant cultures was between 1 and 30% of that in cultures of normal glial cells.

Discussion

The present results show that human fibroblasts and glial cells (astroglia) share a cell surface antigen, SFA, which has not been found in other types of human cells (2). The concentration of SFA per milligram protein is about two to three times higher in cultured glial cells than in fibroblasts. In SFA immunofluorescence all glial cells uniformly stained strongly positive. In analogy with these findings it has been shown that while chicken fibroblast SFA is not detected in several other types of cells, cells grown from explants of chicken brain that have
astroglia morphology are strongly positive for chicken SFA in immunofluorescence (unpublished data).

The presence of SFA in fibroblasts and glial cells but not in other cells suggests that SFA may be associated with their functional similarities such as the presence of cytoplasmic extensions, strong mobility, or role as supportive cells (20). Presence of SFA does not appear to be an expression of a close ontogenic relationship since glial cells originate from neuroectoderm, whereas fibroblasts are of mesoderm origin (21).
Table II
Radioimmunoassay of SFA in Cells and Growth Media of Normal and Malignant Glial Cell Cultures

| Cell culture          | Cell extract, SFA, µg/mg protein | Growth medium, SFA µg/mg protein in cell extract |
|-----------------------|----------------------------------|-------------------------------------------------|
| Normal glia           |                                  |                                                 |
| U-787 CG              | 58.4                             | 332.6                                           |
| U-813 CG              | 81.6                             | 391.3                                           |
| MIRI                  | 71.7                             | 482.3                                           |
| Malignant glia        |                                  |                                                 |
| U-105 MG              | 10.9                             | 118.3                                           |
| U-118 MG              | 8.8                              | 106.5                                           |
| U-251 MG              | 0.7                              | 31.3                                            |
| U-343 MG              | 0.4                              | 2.9                                             |
| U-343 MG-a            | 0.3                              | 12.9                                            |
| Normal fibroblast     |                                  |                                                 |
| WI-38                 | 25.2                             | 88.2                                            |
| WI-38/VA13            | 2.8                              | 59.6                                            |

Confluent cultures were subcultured in a 1:2 ratio using 5 ml of medium per 20 cm². 2 days later, when the cultures were still growing, supernates of the medium and extracts of the cell layers were harvested and assayed for SFA as described in Materials and Methods.

Previous reports showed that tumor virus-transformed fibroblasts whether chicken (3) or human (4) lack SFA on their surface. The present results show that this is true of the glia-glioma system and establish for the first time that lack of surface SFA is not confined to fibroblasts transformed in vitro experimentally by viruses, but is found in at least one type of cells obtained from naturally occurring tumors.

The lack of cell surface SFA in malignant cells is not only due to inhibited synthesis of this protein in malignant cells, as shown by its presence in intracellular form in the cytoplasm of glioma cells (Figs. 4–6) and in the culture media of virus-transformed fibroblasts (4) or glioma cells (Table II). The reason for the failure of malignant cells to retain SFA on the surface is unknown, but it may have to do with the increased lability of the cell membrane of malignant cells, perhaps resulting from enhanced proteolytic activity on the surface of such cells (22).

Summary
Fibroblast surface antigen (SFA) is a high molecular weight protein antigen, first shown on the surface of cultured fibroblasts in fibrillar structures. It is shed to the extracellular medium and also present in the circulation (serum and plasma). Fibroblasts transformed by tumor viruses produce SFA but do not retain it on cell surface.

In this report we show that SFA is also present in cultured nonestablished astroglial cells. The glial and fibroblast SFAs are immunologically indistin-
guishable. Glial cells (three different nonestablished lines) contain more SFA per milligram cellular protein than fibroblasts. SFA was located on cell surface in fibrillar striae that frequently extended out from the cell body. Fluorescence was also found intracellularly in the cytoplasm.

Malignant gliomas (astrocytomas) established to grow in culture from human tumor material produced SFA into the growth medium but had very little (lines U-105 MG and U-343 MG) or no detectable (lines U-118 MG, U-251 MG, and U-343 MG-a) cell surface SFA. In cultures of the glioma cells many cells, in particular those that appeared to be in the telophase stage, stained strongly positive for intracellular cytoplasmic SFA.

These data demonstrate that similar to fibroblasts transformed experimentally by oncogenic viruses, cells grown from naturally occurring human tumors (glioblastomas) produce SFA but lose ability to retain it on cell surface.

We thank Dr. T. Wahlström for the MIRI cell line and Miss Leena Aura, Miss Sirpa Ristimäki, Miss Pirjo Sarjakivi, and Miss Margareta Lindström for skillful technical assistance.

Received for publication 15 July 1975.

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