FIBROBLAST SURFACE ANTIGEN PRODUCED BUT NOT RETAINED BY VIRUS-TRANSFORMED HUMAN CELLS*

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We have recently described a cell type-specific surface (SF) antigen (1) that is deleted in chick fibroblasts transformed by Rous sarcoma virus (2). SF antigen is a major surface component and makes up about 0.5% of the total protein of normal cultured fibroblasts. The antigen is shed from cells and is present in circulation (serum, plasma). The molecular equivalents of both cellular and serum SF antigen are distinct large polypeptides (2, 3), one of which (SF210, mol wt 210,000) is glycosylated and on the cell surface highly susceptible to proteases as well as being accessible to surface iodination. Immunofluorescence and scanning electron microscopy have indicated that in chicken fibroblasts the antigen is located in fibrillar structures of the cell surface, membrane ridges, and processes (4). The external transformation-sensitive molecules found by the lactoperoxidase or the galactose oxidase method in fibroblasts (reviewed in ref. 5) seem to represent one of the SF antigen polypeptides (SF210) of the different species studied. An antigen analogous to the chicken SF antigen is present in human fibroblasts and in human serum (6). We have recently shown (7) that human SF antigen is identical to what has been known as the “cold insoluble globulin” (8) and that it shows affinity towards fibrin and fibrinogen.

We show here that human fibroblasts transformed with simian virus 40 (SV40) lack SF antigen. The transformed cells produce this antigen but it is not retained on the surface of these cells. This creates a major difference between the surfaces of normal and malignant cells.

Material and Methods

Cell Cultures. All cell lines were grown at 37°C in Eagle's basal medium plus 10% fetal calf serum. Two established SV40-transformed human fibroblast lines, WI-38/VA13 (9) and WI-SV40 (provided by Dr. S. Stenman, University of Helsinki) were used. They are known not to produce SV40 virus. The SV40-specific nuclear T antigen (kindly tested by Dr. S. Stenman) was positive in 100% of cells of both lines. The origin of the other cell lines was reported earlier (6).

Antibodies. Anti-SF antisera were produced using a modification of the previously published technique (6). A sheep was immunized with SF antigen solubilized with matrix-bound papain (Enzite-EMA, Miles-Yeda, Rehovot, Israel) from the surface of cultured human fibroblasts. The

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1Keski-Oja, J., A. Vahteri, and E. Ruoslahti. 1975. Fibroblast surface antigen (SF): the external glycoprotein lost in proteolytic stimulation and malignant transformation. Manuscript submitted for publication.
solubilized material was treated with antipapain antibodies coupled (10) to Sepharose 4B particles (Pharmacia Fine Chemicals, Inc., Sweden) to remove papain released from the enzyme preparation, and passed through a Sephadex G-200 (Pharmacia) gel filtration column. The SF antigen containing fractions from this separation were used for immunization. The resulting antisera gave a single precipitation line in immunodiffusion against papain-released material and urea-Triton X-100 extracts (2) from fibroblasts and against human serum. To ensure specificity for SF antigen at the level of immunofluorescence and radioimmunoassay, the antiserum was absorbed with calf serum (a constituent of the cell culture medium) and human leukocytes obtained fromuffy coat. Antibodies were isolated from the antiserum by binding to Sepharose 4B to which normal human serum proteins (including SF antigen) had been conjugated (10) and by subsequent elution of the bound antibodies with 8 M urea (11).

**Immunofluorescence.** The cultures were grown on cover slips, rinsed with phosphate-buffered saline (PBS), fixed with formaldehyde (3.5%, 20 min, room temperature), washed with PBS, treated with acetone (10 min, –20°C), and air dried. SF antigen was demonstrated and localized by direct immunofluorescence using anti-SF-sheep IgG conjugated with fluorescein isothiocyanate (fluorescein/protein molar ratio 2.3) and a Leitz Orthoplan microscope (E. Leitz, W. Germany).

Samples tested for the capacity to inhibit SF antigen immunofluorescence were incubated with the fluorescenated antibody preparation for 60 min at 37°C. SF antigen (cold insoluble globulin; ref. 7) for fluorescence inhibition tests was purified from human plasma by cryoprecipitation, DEAE cellulose chromatography (8), and isoelectric focusing. The preparations gave a single line of precipitation against anti-SF and anti-whole human serum in immunodiffusion and were adjusted to 100 µg/ml of protein for inhibition tests.

**Radioimmunoassay.** Purified SF antigen is not completely soluble in aqueous buffers and we experienced difficulties in trying to set up a radioimmunoassay based on iodinated SF antigen. The assay we used consisted of binding of 125I-labeled (12) anti-SF antibodies to glutaraldehyde-fixed (1.5%, 30 min) fibroblast monolayers on 35-mm plates and inhibition of the binding by SF antigen-containing samples. Serial dilutions of normal human serum were used as standards. SF antigen concentrations are given as relative units where 1,000 U equal the antigen concentration of undiluted standard human serum in the assay.

**Results**

Immunofluorescent staining with anti-SF antibodies revealed that SF antigen had a highly nonrandom fibrillar distribution on the surface of normal human fibroblasts fixed with formaldehyde and acetone (Fig. 1). When live fibroblast cultures were stained at ±0°C and then fixed or when glutaraldehyde-fixed cultures (1.5%, 30 min) were examined, a similar pattern of fluorescence was seen in agreement with the surface localization of the antigen. SV40-transformed cells (either WI-38/VA13 or WI-SV40) had no detectable antigen (Fig. 1). Old dense cultures often had traces of SF antigen located between the cells, but the cells themselves were negative. The staining reactions were totally blocked by purified human SF antigen or normal human serum but not by bovine or chicken serum.

Radioimmunoassay showed the presence of SF antigen in urea-detergent extracts of normal fibroblasts but not extracts of other normal cells or of any of the transformed cell lines tested (Table I).

Radioactive anti-SF antibodies bound to normal cells whereas binding to transformed cultures did not exceed the values obtained with SF antigen-negative (nonfibroblast) cells. The SF antigen content at the surface of normal cultures as measured with this technique was at least 10 times higher per mg cell protein than that in transformed cultures. Immunodiffusion identified SF antigen in urea-Triton X-100 extracts of normal fibroblasts and serum in agreement with our previous observations (7). Extracts of transformed cells
Fig. 1. Immunofluorescent staining for human SF antigen in cultures of normal (left) and SV40-transformed (right) human fibroblasts. Fixation with formaldehyde and acetone.

showed no detectable reaction with anti-SF antiserum. SF antigen was also found by immunodiffusion in concentrated culture fluid samples of normal fibroblasts and also from those of transformed cells (Fig. 2). Quantitation by radioimmunoassay showed that the culture fluids of SV40-transformed cells contained about 30-40% of the SF antigen in fluids of normal cells calculated per mg cellular protein (Table I).

Discussion

These and previous results on chicken, hamster, and mouse (reviewed in ref. 5) fibroblasts show that a major cell surface component is lost when fibroblasts are transformed. The present result on the human fibroblast-SV40 system indicates that both normal and transformed cells produce the antigen but the latter do not retain the antigen on the surface. The phenomenon is not restricted to cell lines transformed experimentally by viruses. Our recent experiments indicate that whereas cultured normal human fibroblasts and glia cells contain SF antigen, established lines of the corresponding malignant cells, sarcomas, and gliomas do
**TABLE I**

**SF Antigen in Extracts of Cultured Human Cells in Culture Media; Inhibition of Binding of ^125^I-Labeled anti-SF Antibodies to Glutaraldehyde-Fixed Fibroblast Monolayers**

| Sample                      | cpm bound* | SF antigen |
|-----------------------------|------------|------------|
| Control                     | 1,010      | 0          |
| Normal human serum          |            |            |
| 100 %                       | 108        | 1,000      |
| 10 %                        | 301        | 100        |
| 1 %                         | 526        | 10         |
| 0.1 %                       | 930        | 1          |
| Calf serum                  |            |            |
| Normal cell extracts        |            |            |
| WI-38                       | 521        | 10         |
| MRC-5                       | 452        | 17         |
| HIM                         | 433        | 21         |
| Amnion (primary)            |            |            |
| WI-38/VA-13                 | 983        | <1         |
| WI-SV40                     | 991        | <1         |
| Amnion WI-SV40              | 1,086      | <1         |
| Amnion U                    | 1,080      | <1         |
| Epithelial (carcinoma)      |            |            |
| HeLa                        | 1,209      | <1         |
| KB                          | 1,115      | <1         |
| Spent culture medium        |            |            |
| Normal fibroblast           |            |            |
| MRC-5                       | 441        | 19         |
| WI-38                       | 480        | 14         |
| SV40-transformed fibroblasts|            |            |
| WI-38/VA-13                 | 605        | 6          |
| WI-SV40                     | 630        | 5          |
| Control medium              | 991        | <1         |

Confluent cultures were subcultured in a 1:2 ratio and assayed after 24 h for SF antigen in the cell extracts and culture media. Cellular SF antigen was solubilized with a solution containing 8 M urea, 1% Triton X-100, 0.02% sodium azide, and 1 mM phenylmethylsulphonyl fluoride (2) for the assays, about 250 µl of extract per culture. All extracts were adjusted to 1 mg cellular protein per ml.

*Mean of duplicate assays on two samples.

**Fig. 2.** Immunodiffusion in agarose. (A) Normal human serum. (B) Concentrated medium from human fibroblast cultures. (C) Human fibroblasts extracted with 8 M urea and 1% Triton X-100. (D) Concentrated medium from SV40-transformed fibroblast cultures. (E) SV40-transformed fibroblasts extracted with 8 M urea and 1% Triton X-100. (F) Fetal calf serum. Center well: sheep anti-SF serum.
not show SF antigen on cell surface, but it is produced to the culture medium (in preparation). Progress in understanding the significance of this change to the transformed state obviously calls for further work.

It would be important to know how SF antigen molecules reach a soluble state when shed from normal cells and why they are not retained at all by the malignant cell surface. It has been shown that transformed cells in culture produce a serine protease that is measured by its ability to convert serum plasminogen to the protease plasmin (13). Proteolysis as the mechanism of the splitting of SF antigen from the cell surface thus is a possibility.

Summary

Normal human fibroblasts contain a cell type-specific glycoprotein antigen (SF) that is known to be slowly shed into the medium and to be present also in human serum. Immunofluorescence with anti-SF antibodies showed that SF antigen has a highly nonrandom fibrillar distribution in surface of normal fibroblasts. Simian virus 40-transformed fibroblasts also produced the SF antigen, as shown by radioimmunoassay or immunodiffusion tests, but it was not retained by the surface of these cells. This creates a major difference between the surfaces of normal and malignant cells.

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