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FIBROBLAST SURFACE ANTIGEN (SF): Molecular Properties, Distribution In Vitro and In Vivo, and Altered Expression in Transformed Cells

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We have recently described a cell type-specific surface (SF) antigen that is deleted in chick fibroblasts transformed by Rous sarcoma virus. SF antigen is a major surface component and makes up about 0.5% of the total protein on normal cultured fibroblasts. The antigen is shed from normal cells and is present in circulation (serum, plasma), and in vivo, also, in tissue boundary membranes. The molecular equivalents of both cellular and serum SF antigen are distinct, large polypeptides, one of which (SF210, MW 210,000) is glycosylated and, on the cell surface, highly susceptible to proteases and accessible to surface iodination. Immunofluorescence and scanning electron microscopy have indicated that the antigen is located in fibrillar structures of the cell surface, membrane ridges, and processes.

Human SF antigen is present in human fibroblasts and in human serum. We have recently shown that human SF antigen is identical to what has been known as the "cold-insoluble globulin" and that it shows affinity toward fibrin and fibrinogen. Our results also indicate that loss of the transformation-sensitive surface proteins is due not to loss of synthesis but to lack of insertion of the protein in the neoplastic cell surface. Both normal and transformed cells produce the SF antigen, but the latter do not retain it in the cell surface.

The loss of SF antigen, a major cell surface component, from malignant cells creates an impressive difference between the surface properties of normal and malignant cells. The possible significance of SF antigen to the integrity of the normal membrane and its interaction to surrounding structures is discussed.

INTRODUCTION

Several lines of evidence implicate changes in cell surface as playing a primary role in oncogenic transformation and the altered growth behavior of malignant cells. Changes in antigenic properties, chemistry of glycolipids, carbohydrate composition of surface glycoproteins, glycosaminoglycans, lectin-effected cell agglutination, glycosyl transferase activity, protease activity, and nutrient transport have been demonstrated (references 1–6). Notably, dissenting reports have appeared on most of these properties (references 7–10). The picture may thus appear confusing in some areas, but it also seems that many of the contradictory data are derived from the comparison of selected transformants to "pseudonormal" rodent cell lines (e. g., 3T3, NIL, BHK) that become aneuploid during

prolonged passage in vitro and that are often tumorigenic themselves (11–13).

Proteins exposed at the cell surface are likely to be critically involved in normal intercellular control and in malignant transformation. It appears that study of these phenomena will require isolation and characterization of the biologically pertinent molecules and that immunochemical methods should provide a useful tool to this end. With these considerations in mind, we have set ourselves to study the conversion of chicken and human fibroblasts to sarcoma cells, two models in which strictly diploid cells may be compared to their virus-transformed derivatives. Selective proteolysis of external cell surface proteins using matrix-bound papain led to the identification of an external major cell surface glycoprotein, fibroblast surface antigen (SF), that is lost from transformed cells (14, 15). A very different approach, by use of surface labeling, lactoperoxidase-catalyzed iodination (16–22), or galactose oxidase-catalyzed tritiation (23, 24), has also demonstrated a large external glycoprotein absent from the surface of virally transformed fibroblasts. Several lines of evidence have indicated that the surface-labeled protein represents one of the SF antigen molecules.

The purpose of the present report is to summarize the information available on the chemical and biological properties of SF antigen molecules. With anti-SF antibodies and with information on the molecular equivalents of SF antigen, we have been able also to study their metabolism and distribution in vitro and in vivo. We will also attempt to consider briefly the possible functions of SF antigen molecules and to relate the findings to other alterations associated with malignant transformation.

ANTI-SF ANTIBODIES AND THEIR SPECIFICITY

Digests of extensively washed live fibroblast cultures were obtained using brief treatment with matrix-bound papain, clarified by ultracentrifugation, dialyzed, and used to immunize sheep or rabbits. After absorption with calf serum (a component of the fibroblast growth medium) and with insolubilized inactivated (iodoacetamide-treated) papain, antisera prepared from the early bleedings of the immunized animals have given a single precipitation line agains the immunogen, concentrated supernatants of spent medium of the fibroblast cultures, and against serum of the species of the fibroblasts. Such data obtained in the chicken (14), human (25), and mouse systems (unpublished observations) first indicated that the antigen we have designated fibroblast surface (SF) antigen, was shed from the fibroblasts into the medium and was also present in circulation (serum, plasma). It is now known that the monospecificity of such seemingly crude anti-SF antisera in immunochemical tests was most probably due to two factors: SF antigen in cultured fibroblasts is a major surface component (about 0.5% of total protein in cultured fibroblasts) and is highly susceptible to solubilization by proteolysis by, e.g., papain, trypsin, and plasmin (22), but relatively resistant to further splitting by proteases (25). To ensure specificity for SF antigen at the level of immunofluorescence and radioimmunoassay, anti-SF antibodies have been isolated from IgG fraction of the antisera by binding to Sepharose 4B to which serum proteins (including SF antigen of the species studied) had been conjugated and by subsequent elution of the bound antibodies with 8M urea (26). In addition, antihuman SF antisera have been absorbed with purified lymphocytes and group AB red blood cells.

The specificity of the antibodies is indicated by the following observations (1) Highly purified human plasma SF antigen ("cold-insoluble globulin," see below) that gives a single precipitation line against antinormal human serum reagents totally blocks immunofluorescence by anti-SF antibodies. (2) Antisera prepared against purified serum SF antigen gave a fibrillar pattern of immunofluorescence in cultured fribroblasts similar to that obtained with anti-SF antibodies directed against fibroblast surface SF antigen. (3) The antibodies are not blocked in immunofluorescence by heterologous sera, and have not reacted with cellular material originating from cells other than fibroblast or glial cells (see distribution of SF antigen below).

Our recent data (Ruoslahti and Vaheri, manuscript in preparation) indicate that immunological cross-reactions can be detected among chicken, Japanese quail, mouse, rat, guinea pig, hamster, and human SF antigens, both in immunofluorescence tests and in immunodiffusion analysis. Anti-human SF antibodies prepared in sheep react not only with SF antigen of various mammalian species but also with chicken SF antigen. Conversely, sheep and chicken SF antibodies react with mammalian SF antigens. Such cross-reactions are commonly detected between homologous proteins, e.g., the oncofetal protein alpha-fetoprotein (27) and the group-specific proteins of C-type viruses (28) of different mammalian species, but only rarely, e.g., in the case of actin (29), between species as far from each other as chicken and man, and suggest that the structure of SF protein has remained highly conserved during evolution.

MOLECULAR PROPERTIES OF SF ANTIGEN

When papain was used to release antigenic material from the surface of cultured fibroblasts, SF antigen was obtained as soluble molecules with apparent molecular weights of about 50,000. We now know that they are cleavage products derived from high molecular weight SF polypeptides.

SF antigen molecules of cultured fibroblasts may be quantitatively solubilized with a solution containing 8M urea, neutral detergent (1% Triton X-100), and protease inhibitor (0.1–1.0 mM phenylmethylsulfonylfluoride) (15). Urea-detergent-extracted SF antigen is immunologically indistinguishable from the papain-cleaved fragment and may be quantitated by radial immunodiffusion or radioimmunoassay.

We have used immunoprecipitation with anti-SF antibodies and immunoadsorbents to isolate SF antigen molecules from urea-detergent extracts of ^{35}S-methionine-labeled fibroblasts. Both isolation procedures have given similar results. The molecular equivalents of chick fibroblast SF antigen are two large polypeptides with apparent MW of 210,000 and 145,000, respectively, SF210 and SF145 (15, 26). When a short, 60-min pulse of ^{35}S-methionine is used to label fibroblast cultures, the SF antigen molecules isolated are demonstrable as the two distinct polypeptides. Polypeptide analysis of SF antigen isolated from cultures labeled for 24 hr shows additional polypeptides, such as SF185. Tryptic peptide analysis of iodinated polypeptides eluted from gel electrophoresis has indicated that SF185 is most probably a proteolytic cleavage product of SF210. In addition to these high molecular weight polypeptides, immunoprecipitates of SF antigen contain a 45,000 MW component that comigrated in electrophoresis (26) with actin purified from fibroblasts. These observations have raised the possibility that an association may exist

between SF antigen molecules and microfilament actin in the surface of normal fibroblasts. SF antigen isolated by similar immunochemical procedures from chicken serum showed a polypeptide composition similar (SF210 and SF145) to that from fibroblasts, except that the 45,000 MW component was not detected (26). From this molecular similarity and the distribution of SF antigen in vivo (see below), we conclude that the serum SF antigen is, in fact, derived from fibroblasts.

Our recent results (unpublished observations) show that the human serum SF antigen closely resembles that of chicken SF antigen in being composed of two chains with MW close to 210,000 and possibly a minor component with MW of about 150,000.

Immunochemical data suggest that the two polypeptide chains, SF210 and SF145, carry the same antigenic determinant. SF antigen behaves as a single antigen-antibody system, giving only one line of precipitation in immunodiffusion (14, 15, 25). The metabolisms of SF210 and SF145 are, however, distinctly different. SF210 is glycosylated, accessible to lactoperoxidase, and highly susceptible to trypsin, whereas SF145 is characterized by a turnover rate uniquely rapid in fibroblasts (15, 22, 26). The role of glycosylation and/or proteolytic cleavage in the relationship of the two polypeptides is being investigated at present.

DISTRIBUTION OF SF ANTIGEN IN CULTURED FIBROBLASTS

SF antigen is synthesized by cultured fibroblasts and is also released into the extracellular space. Quantitative immunochemical assays and examination of the same cells by immunofluorescence and scanning electron microscopy (30) have indicated that SF antigen may be considered to occur in four different forms: (1) intracellularly in the cytoplasm; (2) on the cell surface in association with fibrillar structures; (3) on the growth substrate as a fibrillar network extending outside the cell body; (4) in soluble form in the extracellular medium. The intracellular form is particularly prominent in fibroblasts in the telophase stage of the cell cycle. In cultures of human glia cells (see below), the cytoplasmic "granular" fluorescence of SF antigen is seen uniformly in all cells. In cultures of chick fibroblasts, SF antigen is located predominantly on the cell surface and is localized in membrane processes and cell surface ridges, with a diameter of 50–200 nm. The processes extend from the periphery of the cells to the substratum or to other cells. Trypsin treatment, such as is used in the subculture of fibroblasts, removes all detectable amounts of SF antigen. The antigen is detectable within 1 hr after trypsin-treated cells are reseeded and the reappearance of SF antigen correlates with the restoration of membrane processes.

The amount of SF antigen released to the medium is considerable. For instance, when confluent cultures of chick embryo fibroblasts have been maintained for 24 hr under conventional culture conditions, about one-third of the total antigen is detected in the medium.

An important question is the nature of the exact structural relationship between the cellular and circulating antigen (or the antigen fibroblasts shed to the culture medium). This relates to the question of how the antigen reaches the soluble state. It may be excreted as such or cleaved off from the cell surface by enzymes present in the membrane. Although the polypeptides of the cellular and serum SF antigen seem indistinguishable at the present level of resolution, the size of the polypeptides makes it difficult to exclude

the possibility that they might differ by a peptide segment which could anchor the molecule to the membrane.

Mild proteolytic treatment on normal fibroblasts in culture transiently gives them several properties attributable to transformed cells. The external surface glycoprotein (SF210) is removed (14, 16, 22), glucose transport (31, 32) and agglutinability by lectins (33–36) are increased. Confluent normal cells may be also released from density-dependent inhibition and go through another cell cycle (37, 38).

Our unpublished work (manuscript in preparation) indicates that SF antigen is not detectable on surface of mitotic fibroblasts and the work by Hynes and Bye (39) also shows that in synchronized NIL hamster fibroblasts mitotic cells have low levels of the large external glycoprotein detectable by lactoperoxidase-catalyzed iodination. It has been reported that metaphase cells have a high level of protease activity on the cell surface (40). These data support the hypothesis that cyclic alterations in cell surface proteolysis may be important in normal growth control, as recently discussed (1, 2), and that SF antigen (SF210) on the cell surface may be a critical substrate for the surface proteases (41).

It is known, however, that cell proliferation is initiated in density-inhibited cultures of chick embryo fibroblasts by insulin (22, 42) and thrombin (43) without any detectable early alteration in the external surface glycoprotein (SF210). These data suggest that release of SF antigen is not directly involved in initiation of proliferation from the G_1 (or G_0) phase, but may be an essential feature in mitosis.

DISTRIBUTION OF SF ANTIGEN IN DIFFERENT CELL TYPES AND IN VIVO

The only two cell types in which SF antigen has been so far detected are fibroblasts and glia cells, both considered to be performing a connective tissue function. We have examined five different human fibroblast lines (WI-38, MRC-5, IHM, 377HEL, ER) and several lines of glial cells (787CG, 813CG, MIRI) and have consistently detected human SF antigen in all (25, 44). Chick fibroblasts (14) and some glia-like cells of chick brain cell cultures contain chicken SF antigen. Mouse fibroblasts contain an analogous antigen also present in mouse plasma and serum. Quail embryo fibroblasts, when stained with fluorescein-conjugated anti-chick SF antibodies, show a fibrillar pattern similar to that seen in chick fibroblasts (unpublished observations). Both immunochemical quantitation and immunofluorescence indicate that cultured glial cells contain more SF antigen per mg cellular protein than fibroblasts do (44). We have not detected SF antigen in cell lines derived from other cell types, such as normal or transformed human amnion cells, carcinoma cells (HeLa, HEp-2, KB), erythrocytes, buffy coat leukocytes, and Burkitt's lymphoma cells (25).

The distribution of SF antigen has been studied in some detail in the developing chick embryo (45) by cryostat sections and immunofluorescence. These and previous studies have established that the chick SF antigen is present (1) in loose connective tissue and already in the primitive mesenchymal tissues of very early (2–3-day old) embryos; (2) in limiting membranes throughout the organism, such as the glomerular and tubular basement membranes of the kidney, the boundary membranes of the notochord, yolk sac, and vitelline membranes; (3) in soluble form in circulation (serum, plasma) and

TABLE I. Main Properties of Fibroblast Surface Antigen Molecules

Property	Evidence
Cell type (fibroblast, glia) specific	Immunofluorescence, immunochemical quantitation
Associated with fibrillar structures of cell surface	Immunofluorescence and scanning electron microscopy
Present in circulation. Serum SF antigen = "cold-insoluble globulin." Shed from fibroblasts in vitro.	Immunochemical tests, polypeptide analysis
Present in vivo in connective tissue and tissue boundary membranes	Immunofluorescence
Glycoprotein	Incorporates, e.g., fucose, glucosamine, methionine, and leucine. Binds concanavalin A.
Molecular equivalents: two polypeptides with MW 210,000* (glycosylated, accessible to lactoperoxidase, highly susceptible to trypsin and plasmin) and 145,000 (uniquely rapid turnover)	Gel electrophoresis, immunoadsorbents, radioimmunoassay, pulse-chase labeling experiments
Cellular SF antigen associated with a 45,000 MW polypeptide (comigrating with actin)	Immunoprecipitation and gel electrophoresis
Absent from transformed cells (produced but not retained at surface)	Immunochemical quantitation, immunofluorescence, polypeptide analysis

*This polypeptide (SF210) appears to be the same (22, 39) as that detected and reported by several workers (16–24) using surface labeling in comparison of normal and virus transformed fibroblasts.

For other references see text.

in the allantoic fluid (unpublished observations). These in vivo studies confirm that SF antigen is cell type specific. It was seen as a fibrillar network in the loose connective tissue of different organs but not in the parenchymal cells. It was not found in muscle cells at any stage of development. The antigen was present in the undifferentiated mesenchymal cells of the kidney, but was not found after their development into epithelial cells of the tubules. Both in vivo and in fibroblast cultures, SF antigen was distributed as a fibrillar network.

These data indicate that SF antigen is a "differentiation antigen" restricted to certain cells of mesenchymal origin and character and that it accumulates in the connective tissue during embryogenesis.

SF antigen in cultured fibroblasts is a major surface component and, as shown by the in vivo studies, is widely distributed throughout the connective tissue. SF antigen appears to be one of several fibroblast-derived molecules (collagen, glycosaminoglycans) that form the intercellular matrix. Our recent work has shown that human plasma SF antigen has an affinity to fibrin and fibrinogen (46) and is identical to what has previously been called "cold-insoluble globulin" (47, 48). The significance of the SF antigen-fibrinogen interaction is not known.

SF ANTIGEN IS PRODUCED BUT NOT RETAINED BY TRANSFORMED CELLS

The amount of SF antigen was greatly reduced in amount or absent in fibroblasts transformed by five different Rous sarcoma virus stains. Fibroblasts infected with virus mutants temperature-sensitive for transformation recovered SF antigen when maintained at the nonpermissive temperature. Productive infection with a nontransforming avian type C virus did not alter the level of SF antigen characteristic of normal fibroblasts. These results were obtained with either immunochemical quantitation of SF antigen, polypeptide analysis of cell extracts (loss of SF210 and SF145 in the transformed state; reference 15), or immunofluorescence staining (30)..

These and surface labeling studies on chicken (16, 18–21), hamster (16, 23, 24), and mouse (17) fibroblasts show that a major cell surface component is lost when fibroblasts are transformed by oncogenic viruses. The human fibroblast-SV40 system (49) has indicated that both normal and virus-transformed cells produce the antigen, but that the latter do not retain the antigen on the surface. This result was obtained by immunodiffusion, radioimmunoassay, immunofluorescene (49), and polypeptide analysis (unpublished observations). 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, show little or no SF antigen on cell surface but release it into the culture medium (44). Progress in understanding the significance of this change to the transformed state obviously calls for further work.

It is important to know how SF antigen molecules reach the soluble state when shed from normal cells, and why they are not retained by the malignant cells surface. Although the polypeptide composition of the cellular and serum antigen (26) and, on the other hand, of the antigen produced by normal and virus transformed human cells (unpublished observations) appear similar in conventional gel electrophoretic conditions, the size of the polypeptides makes it difficult to resolve minor chemical differences. 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, which, in turn, is assayed by its ability to hydrolyze fibrin (50, 51). Proteolysis as the mechanism of the splitting of SF antigen from the cell surface thus represents one possibility. It is also important to determine whether increased sloughing off of surface components, as shown here for SF antigen, is true for other surface molecules in malignant cells.

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