Cell Cycle Dependency of Tumor Antigens

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Summary

There is now substantial evidence that suggests that tumor antigen expression is cell cycle dependent. This information has been obtained from a wide variety of tumor systems. The G phase of the cell cycle has been implicated by a number of investigators as the point of maximal antigen appearance. In this presentation, we are concerned with a human sarcoma-associated tumor antigen and its cell cycle-dependent appearance. Sarcoma-associated tumor antigen is a membrane antigen present on cultured human neurosarcoma cells (T2-cells). Although the underlying mechanisms responsible for cell cycle-dependent expression are unclear, several possible interpretations are offered.

Introduction

One of the most perplexing challenges now facing tumor immunologists lies in understanding the nature of tumor antigens. Although the concept of specific tumor antigens is now considered valid (9, 12, 14, 15, 21), the physicochemical properties of tumor antigens are not fully elucidated and the biosynthetic events responsible for their appearance are not clearly delineated. Temporal fluctuations in the expression of these tumor antigens have, in fact, been detected but are presently unexplained (5, 13, 17).

This report examines SATA expression during the transit of T2-cells through the proliferative cycle. The finding of cell cycle-dependent expression coincides with findings in other macromolecular systems including collagen (10), myosin (16), immunoglobulins (4), proteins necessary for the initiation of DNA synthesis (6, 26), and plasma membrane proteins (11).

Several interpretations for the observed fluctuations in SATA membrane appearance are pertinent, including unmasking of antigenic sites on the cell surface, configurational changes in the membrane, de novo synthesis of antigenic determinants, and others which will be discussed.

Materials and Methods

Target Cells. The cells utilized as target cells in this study (T2-cells) were derived from a human neurosarcoma in April 1966 (27) and have been maintained continuously in Ham's F-10 tissue culture medium since that time. The cells have been routinely monitored for pleuroneumonia-like organism contamination by biochemical (1) and microscopic means.

Kinetic parameters for T2-cells are: cell cycle time, 58 hr; G phase, 27.5 hr; S phase, 22 hr; G2 phase, 8.5 hr; doubling time, 63 ± 3 hr.

Cell Synchrony Procedures. T2-cells were synchronized by a combination excess TdR block (4 mM TdR for 48 hr to obtain S and G2 phase cells) followed by mitotic selection (for M and G1 phase cells). The degree of synchrony achieved with the TdR block was monitored by pulse-labeling with [3H]CdR (1 μCi/ml; 9.59 Ci/m mole) followed by an autoradiographic assessment of the percentage of labeling index. Synchronization by mitotic selection was monitored by assessing the mitotic index initially, followed by labeling index determinations with [3H]CdR throughout G1.

Serum Screenings and Absorptions. A total of 94 serum samples were obtained from various donors. Test samples were obtained from sarcoma patients (33 cases), control patients with nonsarcomatous neoplasias (37 cases), and normal donors (24 cases). Individuals with any previous history of transfusion, pregnancy, or antitumor therapy were excluded from this study. All sera were inactivated for 30 min at 56° and were stored at −20°.

Each serum sample was screened for the presence of antibody to the SATA on T2-cells by indirect immunofluorescence. The cells were incubated for 30 min at 37° with 100-μl aliquots of the test sera, control sera, or 0.9% NaCl solution, followed by 3 washes in fluorescent-tagged antibody buffer; (Difco Laboratories, Detroit, Mich.). The samples were then incubated for 30 min at 37° with fluorescein isothiocyanate-labeled goat anti-human immunoglobulins, washed 3 times in fluorescent-tagged antibody buffer, and mounted in Sorenson's buffered glycerol, pH 8.2, for observation.

Samples were counted as unknowns by 2 independent observers. In the initial screenings, only those sera that gave an average level of 25% fluorescent cells, or greater, were considered positive (7). All positive sera were then absorbed with various control cell panels (5.0 × 106 cells/ml serum) which included human erythrocytes (ABO, Rh positive), established lines of human lymphoma (28), adenocarcinoma of colon (8), melanoma (22), skin fibroblasts, and peripheral nerve tissue obtained from cadavers. These absorptions were performed to rid the samples of nonspecific, cross-reactive antibodies. Sera were rescreened against T2-cells following each absorption.

Immunokinetic Studies. T2-cells were synchronized by the techniques described above and subsequently examined at regular intervals during each of the cyclic phases for
SATA expression by indirect immunofluorescence. Four sarcoma sera with high titers of anti-SATA activity in asynchronous screenings (average, 52% fluorescent cells) were selected. Controls included normal serum samples and 0.9% NaCl solution controls. The degree of synchrony was monitored by incorporation of [³H]Cdr throughout the cell cycle analysis.

Results

Of the 94 sera initially screened for anti-SATA activity, 30 (32%) were positive (Table 1). This included 21 of 33 from the sarcoma group, 8 of 37 from the nonsarcomatous neoplasia group, and 1 of 24 from the normal blood bank donor group. The range in percentage of fluorescent T₂-cells in these initial screenings was from 25 to 60%. The 30 sera that were initially positive were then subjected to sequential absorptions with the cellular panels indicated in Table 2. Results from these absorptions and rescreenings (Table 2) indicate the successful exclusion of serum activity directed against cross-reacting cellular antigenic determinants. Nineteen of the 21 positive sarcoma sera remained positive (i.e., >25% fluorescent cells) following absorption, as did the 1 positive blood bank donor. However, all 8 nonsarcoma neoplasia sera were eliminated following absorption with RBC, lymphoma, and adenocarcinoma cells. The adenocarcinoma cell line used for absorption was derived from a human colon carcinoma and is known to produce substantial amounts of carcinoembryonic antigen (52 ng/ml) (8).

In addition to the exhaustive absorption procedures used, those sera that remained positive postabsorption were tested further for cross-reactivity directed against RBC panels of known phenotypic characteristics (Rh, MN, Duffy, Lewis, Kidd, Lutheran, and sex-linked blood group systems).

Four sarcoma sera, selected from the battery of absorbed sera, were used to assess the cell cycle dependency of SATA expression. Negative controls were normal serum samples and 0.9% NaCl solution. Synchronized T₂-cells were examined by indirect immunofluorescence in each of the cyclic phases for SATA expression. It is readily apparent from Chart 1 that SATA expression is indeed a cell cycle-dependent phenomenon. The 25% fluorescent cells detected in the beginning stages of S phase decreased steadily to negligible amounts (4%) by mid-G₂ phase. SATA expression increased sharply in M phase and reached a peak at the approximate midpoint of G₁ phase (11.5 hr). The percentage of labeling index is indicative of the high degree of synchrony maintained throughout the duration of the cell cycle analysis.

Discussion

The battery of antisera used in this study were carefully prescreened and meticulously absorbed to avoid the possibility of nonspecific (i.e., non anti-SATA) cross-reactivity. The detection of SATA on T₂-cells, with 19 of 21 postabsorption sarcoma sera, is indicative of the highly specific nature of the observed reaction. The behavior of the sole blood

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**Table 1**

<table>
<thead>
<tr>
<th>Serum sample class</th>
<th>No. positive/total no.</th>
<th>% positive from each class</th>
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<td>Sarcoma</td>
<td>21/33</td>
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<tr>
<td>Non-sarcomatous neoplasias</td>
<td>8/37</td>
<td>22</td>
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<td>Blood bank donors</td>
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<td><strong>Total</strong></td>
<td>30/94 (32% positive sera from initial screenings)</td>
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**Table 2**

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<tr>
<th>Serum group</th>
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<th>Range</th>
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<td>39-44</td>
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<td>41-46</td>
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<td>Blood bank donors</td>
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Somatic cell mutations and the activation of repressed genes represent intrinsic mechanisms for tumor antigen production. Mutational events may give rise to additions or deletions of gene-related products. Perhaps the deletion of gene-related components of the cell surface results in some "compensatory shift" of other components, the end result of which is acquired antigenicity, unique to the tumor cell. If SATA components represent structural additions or deletions of such products due to somatic cell mutations, then cyclic variation of expression is attributable to genetic control mechanisms. Similarly, repressed gene activation is wholly dependent upon gene control mechanisms. Several outstanding examples exist, including the appearance of carcinoembryonic antigen coincident with human colon carcinoma, originally discovered by Gold (9), and the expression of the thymus leukemic antigen in mice which do not normally express this component in any other tissue or at any other time (3). Derepression of genes suppressed since the embryonic stage of life is a possible explanation for these observations. By analogy, SATA may represent the end product of an activated silent gene which is transcribed during a specific interval of time.

Various changes in the properties of membrane components have been correlated with the position of cells in the cell cycle. Structural alterations in the cell surface of transformed CHO cells were examined with scanning electron microscopy by Porter et al. (19). The blebbed appearance of G1 cells was in marked contrast to the flattened appearance of cells in S and G2 phases. Shoham and Sachs (23) and Smets (24) observed cyclic variations in the mobility of membrane receptors. Concanavalin A receptor sites were more readily clustered in the early interphase of transformed fibroblasts and in the mitotic phase of normal fibroblasts. By comparison, SATA components may assume the morphological appearance of blebbed excrescences [similar to those reported by Porter et al. (19)], which are quantitatively most abundant in mid-G1 phase. Perhaps SATA components become mobilized and cluster during G1 phase in a manner analogous to concanavalin A receptor sites in transformed cells. Such a phenomenon would lend itself to maximum detectability.

Membrane components (e.g., carbohydrate moiety commonly encountered at the cell surface) could serve to mask antigenic interactions with the corresponding antibody. Thus, the appearance of SATA could be governed by masking and unmasking on the surface of T2-cells. The appearance of the neoantigen of fibrinogen has been shown to be dependent upon the availability of carbohydrate cleavage enzymes (18).
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In summary, SATA was detectable in all phases of the cell cycle but was significantly greater in G, than at any other point in the cycle. As mentioned previously, the exact mechanism responsible for this sequential expression is not known. However, future studies based upon this information will hopefully lead to a clearer understanding of the physicochemical nature of SATA and of the mechanism(s) controlling its expression.

References

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