Lectin-binding Affinities of Human Epidermal Tumors and Related Conditions


Louis, Chris, J., Wyllie, Robert G., Chou, Sheung To, and Sztynda, Tamara: Lectin-binding affinities of human epidermal tumors and related conditions. Am J Clin Pathol 75: 642-647, 1981. Paraffin-embedded sections from a variety of epidermal lesions were stained with fluorescein iso-thiocyanate-labeled concanavalin A and examined by a fluorescence microscope. Seventy-six normal, hyperplastic, and neoplastic tissues were examined. Lectin binding was demonstrated in all malignant tumors, the fluorescence being confined to the plasma membrane of the tumor cells. Normal and hyperplastic tissues either failed to stain or showed a grossly diminished level of fluorescence. The distinction between malignant and normal or hyperplastic cells was clear-cut and definite. (Key words: Lectins; Plasma membranes; Malignancy; Fluorescence; Diagnosis.)

THERE ARE NO absolute criteria in histopathology for the recognition of malignant cells. Special stains and a variety of physical techniques have narrowed the limits of the problem without answering definitively the central question. The need for a clearly discriminating stain in cases of hyperplasias of the breast, polyps of the intestine, and various hyperplasias of the skin, to mention only a few, has been recognized for years. Such conditions frequently present histologic problems that significantly impinge upon the choice of treatment.

All current methods of staining are inadequate. Nucleus is clearly differentiated from cytoplasm, and various cytoplasmic secretions may be rendered recognizable, but slight differences in the structure of the cell are not demonstrable. The search for methods depending on serologic differences between malignant and normal cells has also been unsuccessful. Experience, the most valuable tool in histopathology, is not always available.

A new approach to this problem has been suggested by the observation that certain plant extracts (lectins) will agglutinate tumor cells but not their normal counterparts.1,2,6,9,11-13 Among the lectins, wheat germ agglutinin and concanavalin A (con A), obtained from jack beans, have been found most effective. They agglutinate all types of tumor cells regardless of whether the tumor arose spontaneously, by chemical initiators, by viral agents, or by ionizing radiations.11,13,15 Furthermore, it has been shown that in cells transformed by temperature-sensitive mutants of oncogenic viruses, the integrity of the viral genome is important in the maintenance of lectin agglutinability.7 Lectins also agglutinate normal erythrocytes and some lymphocytes,11,14,15 but in general, the correlation between lectin agglutinability and neoplasia is very strong.8

Lectins have multiple binding sites for glycoproteins on tumor cell surfaces11 with which they react to form cell aggregates similar to those in the development of lattice formation in precipitating antibody.10 In normal cells, lectin receptor sites are uniformly distributed on the surface, but these sites are normally protected or covered by some component of the plasma membrane. This membrane component can sometimes be modified by light trypsinization.3 In contrast, the receptor sites in malignant cells become clustered in the plasma membrane, and when treated with lectin, the cells agglutinate.11,15,17 These studies suggested that the glycoprotein receptors on the surface of tumor cells differed from normal cell surface glycoprotein.3,14 More recent work by Bramwell and Harris4 has shown that tumor cell glycoprotein is dimeric, while normal cell glycoprotein is monomeric. They believe this glycoprotein receptor to be a component of the glucose transport system of the cell.

These findings and the fact that the cell surface plays a central role in cell division16,17 suggested a possible means of approach for the histologic recognition of neoplastic cells. In a preliminary study, we examined histologic sections from a variety of human tumors (breast, lung, liver, pancreas, colon, muscle, and connective tissue) by using fluorescein isothiocyanate-labeled concanavalin A (FITC-con A) as a histochemical stain. In all cases, the conjugate became bound to the plasma membrane of the tumor cells. Binding was demonstrated by a bright-green rim of fluorescence. The
nonneoplastic parent cells, on the other hand, either failed to bind the conjugate or showed grossly diminished binding. To decide whether this gross difference in binding affinity for FITC-con A is a peculiarity of malignant cells, or whether it is an expression of rapidly proliferating cells, we embarked on a general investigation of the staining characteristics of human tumors. In this paper, we describe the results obtained in normal and hyperplastic conditions, and in benign and malignant tumors of the epidermis.

Materials and Methods

The tissues examined were obtained from surgical specimens received during the course of an operation. All tissues were fixed in a 10% solution of formal saline buffered to pH 7.0. Fixed tissues were processed and embedded in paraffin by routine laboratory methods, and from each block of tissue, sections were cut serially at 4-μm thickness and mounted on cleaned histologic glass slides. Following deparaffinization in xylene, the sections were brought to water through alcohol and were ready for staining.

Procedure

The direct fluorescence staining technic was used to determine binding of lectin to plasma membrane. The tissue sections were stained for 30 min with a few drops of FITC-con A diluted in 0.08 M phosphate-buffered saline solution (PBS), pH 7.3, to a concentration of 0.015 mg/ml in a moist chamber, and then gently washed in three changes of PBS.

Fluorescence Microscopy and Photography

A Leitz Ortholux® fluorescence microscope fitted with a HBO 200W mercury lamp, Ploem® epillumination, and an Orthomat® fully automatic microscope camera was used. Tri-X film (Kodak) was used for photography. Appropriate areas were selected and photographed under ultraviolet (UV) light, then the sections were washed in PBS and stained with hematoxylin and eosin, and the same areas were rephotographed under visible light for comparison with the photographs taken under UV light.

Results

The epidermal conditions examined in this study are shown in Table 1. Positive staining with FITC-con A showed up as the characteristic bright-green fluorescence of fluorescein. Primary autofluorescence in tissue was identified by first examining unstained mounted sections in UV light. Keratin emitted a blue-white fluorescence, while elastic tissue emitted a linear yellow fluorescence.

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Cases</th>
<th>Fluorescence†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal skin</td>
<td>8</td>
<td>−</td>
</tr>
<tr>
<td>Hyperplasias</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperkeratotic warts</td>
<td>4</td>
<td>−</td>
</tr>
<tr>
<td>Keratoacanthoma</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>Irritated epithelium</td>
<td>7</td>
<td>−</td>
</tr>
<tr>
<td>Nevus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junctional</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>Compound</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>Intradermal</td>
<td>6</td>
<td>−</td>
</tr>
<tr>
<td>Blue</td>
<td>2</td>
<td>−</td>
</tr>
<tr>
<td>Premalignancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solar keratosis</td>
<td>8</td>
<td>( + )</td>
</tr>
<tr>
<td>Bowen's disease</td>
<td>12</td>
<td>−</td>
</tr>
<tr>
<td>Hutchinson's melanotic freckle</td>
<td>4</td>
<td>−</td>
</tr>
<tr>
<td>Malignancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>12</td>
<td>++</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>10</td>
<td>+++</td>
</tr>
</tbody>
</table>

† The staining observed was a rim of green fluorescence on the surface of the tumor cells: (+, ++, +++ = degrees of brightness, (−) = absence of fluorescence.

Normal and Hyperplastic Tissues

Squamous cells, basal cells and nevus cells in normal epidermis (Fig. 1), hyperkeratotic warts, "irritated" hyperplastic epithelium at the margin of benign ulcers and epidermal infections, and keratoacanthomas did not stain.

Nevi

Of the 16 examples of nevi that were examined, three were junctional, five compound, six intradermal, and two blue nevi. All the proliferating nevus cells, whether single or in clusters, whether or not they contained pigment, and whether they were located at the dermoepidermal junction or within the dermis, were negative for fluorescein fluorescence (Fig. 2).

Carcinoma

In all clearly malignant tumors, most of the tumor cells bound FITC-con A conjugate and emitted bright-green fluorescence that was confined to the surface of the cells. Occasionally, only part of the cell membrane stained, forming an arc or cap of fluorescence. The cell cytoplasm and nucleus did not stain. The types of epidermal cancers examined are shown in Table 1. Squamous cell carcinomas ranged from well differentiated with prominent intercellular prickles and many keratin
pearls to poorly differentiated with atypical squamous cells lacking prickles and keratinization. Most of the tumor cells showed a rim or cap of fluorescence (Fig. 3). As in normal skin, keratin pearl autofluorescence was pale blue, and elastic tissue was yellow. In basal cell carcinomas, the tumor cells also bound FITC-con A, but to a lesser extent than the cells of squamous cell carcinomas. Fluorescence was always brightest in cells at the edge of the cell groups and least in cells situated within the body of the cell groups (Fig. 4). Malignant melanomas showed the brightest fluorescence of all epidermal tumors (Fig. 5). The fluorescence was not quenched or damped by the presence of melanin pigment.
FIG. 3 (upper). Paraffin section prepared from a poorly differentiated squamous cell carcinoma of the skin. A (left) Fluorescence photomicrograph after staining with FITC-con A and showing fluorescing tumor cells. B (right) Same area as shown in A after washing in PBS and staining with hematoxylin and eosin. All the tumor cells fluoresce—the fluorescence is confined to the cell surface of tumor cells. A small nest of squamous tumor cells (upper right) shows enhanced fluorescence due to the presence of keratin. ×200.

FIG. 4 (lower). Paraffin section from the invading edge of a basal cell carcinoma. A (left) Fluorescence photomicrograph after staining with FITC-con A and showing an irregular sheet of fluorescing tumor cells. B (right) The same area as shown in A after washing in PBS and staining with hematoxylin and eosin. Malignant basal cells show cell membrane fluorescence, which is particularly prominent at the free margin of the advancing edge of tumor cells. ×200.

Premalignant Condition and Early Malignant Change

Solar keratosis, a common condition of the exposed skin surfaces, transforms into skin cancer in 20% of cases. In well-organized lesions, little or no fluorescence was seen. As cellular architecture became disorganized, significant levels of fluorescence occurred in the atypical cells and became maximal at the sites of basement membrane invasion. Thus in preinvasive
FIG. 5 (upper). Paraffin section prepared from a malignant melanoma of the skin. A (left) Fluorescence photomicrograph after staining with FITC-con A, showing bright fluorescence of tumor cells and an area of nonfluorescence (top left corner); B (right) the same area as shown in A after washing with PBS and staining with hematoxylin and eosin. The fluorescing cells are the tumor cells, and the nonfluorescing area represents normal overlying epidermis. × 160.

FIG. 6 (lower). Formalin-fixed paraffin section prepared from the margin of an area of solar keratosis. A (left) Fluorescence photomicrograph after staining with FITC-con A and showing fluorescing and nonfluorescing areas. B (right) Same area as shown in A after washing in PBS and staining with hematoxylin and eosin. Only atypical basal cells fluoresce: the lower group (lower arrow) are superficial to the epidermal basement membrane, the adjacent group of fluorescing cells (upper arrow) have invaded through the basement membrane and extended for a short distance into the superficial dermis. The normal stratified squamous epithelium (upper left) and the dense inflammatory infiltrate in the dermis do not fluoresce. × 200.

Lesions, bright-green membrane fluorescence was found in atypical but not morphologically malignant cells (Fig. 6). In the examples of Bowen’s disease and Hutchinson’s melanotic freckle that were examined here, no lectin binding was detected, but since these lesions are known to have a high incidence of malignant change, further study is necessary.

Discussion

The clear distinction between malignant and non-malignant epidermal cells found in this study is of considerable diagnostic significance and requires further confirmation in a larger series of cases. The results obtained in 32 carcinomas and melanomas and in a
variety of nonmalignant lesions have been unambiguous and consistent. The staining reaction occurs at the cell surface and appears to be specific for receptors on the membranes of malignant cells. Malignant cells bind FITC-con A in significant amounts, whereas nonmalignant cells do not. In this study, our observations were restricted to human epidermal tumors, but the method has been applied to other naturally occurring human tumors. The uniformity of our results indicates a need for a thorough study of neoplastic conditions in man. Skin tumors were considered in this study because of their frequency and importance in this country.

It is well known that many tissues have a natural fluorescence of their own (autofluorescence). Throughout this study we have appreciated that primary intrinsic autofluorescence must be clearly distinguished from the secondary extrinsic fluorescence of the bound fluorochrome. The distinction between these two was achieved by use of adequate controls.

Differential staining appears to be due to binding of the lectin conjugate to a specific receptor in a manner similar to antigen-antibody reactions, with the receptor in this case being a glycoprotein that is not inactivated by formalin fixation and embedding in paraffin. It is not yet established whether this assumption is generally true, or whether there are certain exceptions. So far, none have been found; however, the possibility of inactivation of the receptor by the methods of preparation should be kept in mind. In the past, studies using fluorescein-labeled antibodies showed that while some antigenic sites were inactivated by these procedures, others were not.

Unquestionably malignant skin tumors in this series showed positive staining. Malignant melanomas gave the most striking results, and basal cell carcinomas stained less strongly than squamous cell carcinomas. However, in basal cell carcinomas the level of staining on the free margin of the peripheral layer of cells at the invading edge was always stronger than on cells within the body of the tumor cell masses, suggesting that the invasiveness of malignant cells might in some way be associated with a high density of specific membrane glycoproteins.

The difficulties in distinguishing neoplastic conditions from those that morphologically mimic neoplasms are well recognized by pathologists. That the keratoacanthomas have only recently been segregated from true neoplasms of the skin is a clear indication of this difficulty. The present study indicates that they are benign, but since some have been shown clinically to be malignant, continued scrutiny of these growths is necessary. The distinction between some actively growing nevi and malignant melanoma is also difficult. All examples of actively growing nevi studied here uniformly failed to stain.

While many characteristics of tumor cells have been described, none has been found to be peculiar to the neoplastic state. The pathologist has always had to resort to the general architecture of the tissue for a true assessment of the condition. Here, however, we have a clearly defined feature that distinguishes malignant cells from their nonmalignant counterparts. The technique can be applied to small fragments of tissue as well as to single cells. Its value in the elucidation of the nature of neoplastic and potentially neoplastic conditions needs no emphasis. Furthermore, since the staining reaction is a result of binding to specific receptors on the surface of malignant cells, the method of study opens up possible new avenues of approach to selectively attacking and destroying cancer cells.

References