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LECTIN BINDING AFFINITY IN NORMAL SKIN AND BENIGN, PREMALIGNANT AND MALIGNANT CUTANEOUS LESIONS

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ABSTRACT

The binding sites and affinities of the plant lectins concanavalin A (Con A) and Ricinus Communis Agglutinin (RCA-I) using the avidin-biotin-peroxidase complex (ABC) technique were studied in normal skin, benign, premalignant, and malignant epithelial skin tumors.

The reaction patterns of Con A and RCA-I are similar. The binding sites are located on epithelial cell membranes. The binding affinity increases with cellular differentiation. In normal skin, the binding affinity increases with epithelial maturation, the strongest reactivity occurring within the stratum granulosum. The basaloid cells of seborrheic keratosis are mildly reactive. In actinic keratosis, the reactivity is present in areas of dyskeratosis, but is absent in atypical rete ridges. Bowen's disease and basal cell carcinoma lack reactivity. The reaction pattern of squamous cell carcinoma is variable, increasing proportionately with squamous differentiation.

Lectin binding affinity is a marker of cellular maturation, increasing proportionately with squamous differentiation. The basaloid cells of normal skin and benign basaloid skin tumors have a weak binding affinity which is lost in dedifferentiating premalignant and malignant basaloid skin tumors.

INTRODUCTION

Lectins are glycoproteins with binding affinity for specific carbohydrates. Concanavalin A (Con A) is specific for B-D-mannose, and Ricinus Communis Agglutinin (RCA-I) is specific for β-D-galactose (1).

Carbohydrates are a dynamic part of cell membranes which change with cellular differentiation, maturation, and neoplastic transformation. Several studies have shown an increased binding affinity of lectins in neoplastic cells.

Our study demonstrates that lectin binding affinity varies proportionately with epithelial maturation and squamous differentiation. We speculate that this correlates with increasing concentrations of specific oligosaccharides produced in maturing squamous cell membranes. Therefore, lectin binding affinity can be used as a marker to demonstrate squamous differentiation in epithelial skin tumors.

We have studied the binding sites and affinities of Con A and RCA-I in 5 normal skin controls, 7 seborrheic keratosis, 4 actinic keratosis, 6 Bowen's disease, 9 squamous cell carcinoma, and 5 basal cell carcinomas.

MATERIALS AND METHODS

Blocks of paraffin-embedded tissues were sectioned at 3 μm. After deparaffinization, sections were treated with 0.075% HCl in absolute ethanol to supress endogenous peroxidase activity (2). Sections were treated with a solution of biotinylated lectin (Vector, Burlingame, CA), 10% bovine serum albumin (3) and 0.05 M Tris-buffered saline, pH 7.6 (room temperature) for 30 minutes. Concentrations of biotinylated lectins were: Con A, 10 μg/ml; RCA 50 μg/ml. The Con A solution contained 1 mM $MnCl_2$. Negative controls were produced by addition of 1% of the respective specific monosaccharides to the lectin solutions. This was followed by treatment for 60 minutes with avidin-biotin-peroxidase complex (Vector). It was prepared 30 minutes before use according to the manufacturer's instructions in tris-buffered saline with 0.2% albumin. All steps were followed by washing in Tris-buffered saline. The peroxidase label was visualized by addition of 0.04% diaminobenzidine hydrochloride in ammonium acetate-citrate buffer containing 0.0075% H_2O_2, for 2.5 minutes at pH 5.5 (4). After thorough washing in water, sections were counterstained with hematoxylin and mounted in Permount.

RESULTS AND DISCUSSION

A positive reaction was evidenced by a brownish color on cellular membranes visualized by light microscopy. The staining intensities were graded as negative, mildly, moderately and strongly reactive. Both Con A and RCA showed the same binding patterns. In normal skin, the basal layer was negative to mildly positive. The staining intensity progressively increased with cell maturation, the strongest reaction occurring within the stratum granulosum (Fig. 1). A similar pattern was observed in the follicular epithelium. Sebaceous glands were strongly positive. The acinar epithelium of eccrine glands was negative with a moderate staining reaction in eccrine ductal epithelium.

In seborrheic keratosis, the areas of basaloid acanthosis showed mild reactivity with both Con A and RCA-I. The pseudohorn cysts were moderately positive (Fig.

2). The dyskeratotic cells of actinic keratosis were moderately to strongly reactive; the atypical basaloid cells within the rete ridges were negative to mildly positive (Fig. 3).

Bowen's disease showed a complete lack of reactivity throughout the entire epidermal thickness. In cases with areas of parakeratosis, a moderate reactivity was observed.

In basal cell carcinoma, the basaloid nests were completely negative for RCA but focally positive with Con A. Areas with evidence of squamous or follicular differentiation were moderately to strongly reactive with both Con A and RCA-I (Fig. 4).

Variable staining patterns occurred within infiltrating squamous cell carcinoma which correspond to cellular maturation. In poorly differentiated areas, the reaction was negative to mildly positive, while a strong reaction pattern was observed in dyskeratotic cells and keratin pearls.

Selective binding of lectins to cellular components is determined by the presence of oligosaccharides specific to each lectin. Concanavalin A (Con A) binds specifically to α-D-mannose or α-D-glucose; Ricinus Communis Agglutinin (RCA-I) binds specifically to β-D-galactose.

Epithelial binding of Con A has been investigated more extensively than other lectins. Most of the reports demonstrate a significant increase in its affinity to a wide range of epithelial tumors including carcinoma of skin and melanoma (6), gastric carcinoma (7), endometrial carcinoma (8), and cervical carcinoma (9). However, most of the information concerning human tissues has been derived from the study of formalin fixed paraffin embedded tissue sections (8, 5, 10). These sections show a reduced staining reactivity as compared with frozen section tissue (11).

Our study strongly demonstrates that an increase in staining intensity for lectins by the avidin-biotin-peroxidase complex method in normal skin as well as skin tumors corresponds to the degree of cellular differentiation and probably to the presence of keratohyalin granules, keratinization, and increase in specific oligosaccharides in epithelial membranes.

We conclude that lectin binding affinity in normal skin corresponds to epithelial maturation with the strongest affinity occurring within the stratum granulosum. With epidermal dysplasia and lack of cellular maturation (as in Bowen's disease, actinic keratosis and basal cell carcinoma), the lectin binding affinity is lost. However, in infiltrating squamous cell carcinomas, lectin binding affinity increases with squamous differentiation.

The basaloid areas of seborrheic keratosis show mild to moderate staining reactions which corresponds to proliferation of the maturing cells of the stratum spinosum.

While lectin binding affinity cannot be used as a marker for epithelial tumor identification, it can be used to demonstrate cellular maturation and squamous differentiation within skin tumors, and to better assess areas of epithelial dysplasia in skin biopsy specimens.

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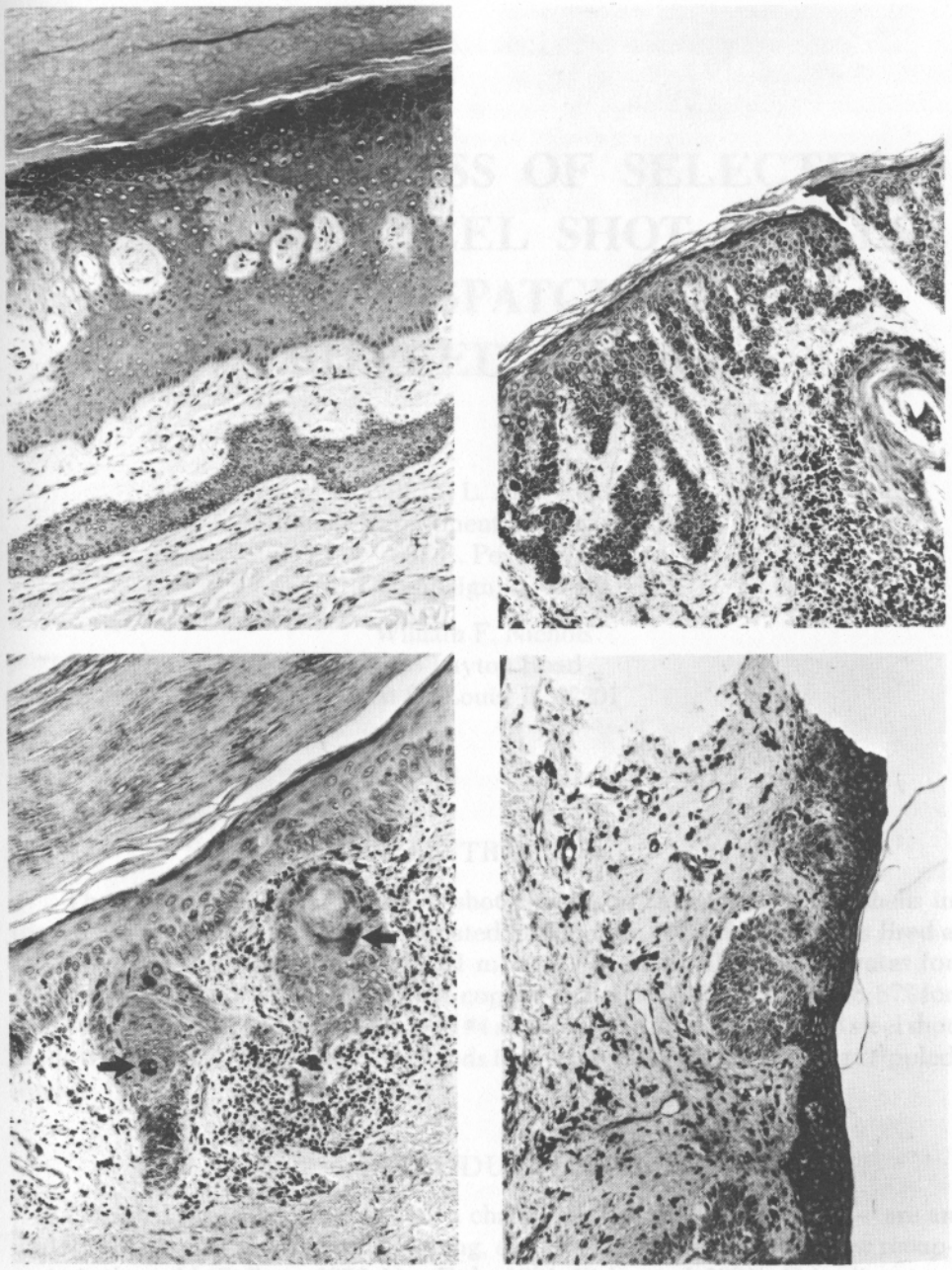

Figure 1. Normal skin: marked reaction for RCA-I in stratum granulosum (Avidin-biotin-peroxidase complex, X 140).

Figure 2. Seborrheic keratosis: mild to moderate reaction in basaloid areas (RCA-I, Avidin-biotin-peroxidase complex, X 140).

Figure 3. Actinic keratosis: lack of positive reaction in atypical areas; positive reaction in dyskeratotic cells (arrows). (Con A, Avidin-biotin-peroxidase complex, X 140).

Figure 4. Basal cell carcinoma: lack of reaction in basaloid nests (Con A, Avidin-biotin-peroxidase complex, X 140).