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Immunohistochemical Study on Differentiations of Carcinomas of the Skin Using Antikeratin Monoclonal Antibodies

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Summary. **Keratins expressed in carcinomas of the skin were immunohistochemically investigated using unfixed frozen specimens and several antikeratin monoclonal antibodies with different specificities. Only simple epithelial keratins were diffusely expressed in Merkel cell carcinomas, mammary and extramammary Peget's diseases, mucinous carcinomas of the skin, and cutaneous metastasis from gastric carcinomas, although the subtypes of simple epithelial keratins were somewhat different among these carcinomas. On the other hand, both simple and stratified epithelial keratins were variously expressed in squamous cell carcinomas, Bowen's disease, basal cell epithelioma and eccrine adenocarcinomas, although considerable differences in the staining patterns were observed in these carcinomas.**

It is suggested that the keratin expressions in carcinomas of the skin may imitate those in the corresponding epithelia of normal skin, although the contents of the keratins of the carcinomas are not always the same as those in the normal epithelia. Immunohistochemical examination seems to be of great value for the differential diagnosis of carcinomas of the skin.

INTRODUCTION

Carcinomas of the skin have been classified into several categories based on their histopathologic and clinical characteristics. Some carcinomas are considered to originate from the epidermis, whereas other carcinomas differentiate toward skin appendages such as hair tissue, sebaceous glands, eccrine and apocrine sweat glands.[1] Such adnexal differentiations of carcinomas have been further confirmed by electron microscopy, histochemistry, enzyme-histochemistry and immunohistochemistry.

Keratins are heterogeneous proteins that constitute epithelial intermediate filaments. Normal epithelial tissues express different subsets of keratins according to their differentiations.[2-4] The differences have been revealed both biochemically by two-dimensional polyacrylamide gel electrophoresis and immunologically by specific antikeratin monoclonal antibodies. In normal human skin, the epidermis and skin appendages have been known to express different keratins according to their differentiations.[5] The analyses of keratins should therefore prove useful in investigating the differentiations of skin tumors.[6]

In the present study, unfixed frozen specimens obtained from several carcinomas of the skin were immunohistochemically investigated with antikeratin monoclonal antibodies in order to determine the characteristics of their keratins.

MATERIALS AND METHODS

Specimens were obtained from the lesions of sixteen cases of squamous cell carcinoma (SCC), fifteen cases of Bowen's disease, twelve cases of basal cell epithelioma (BCE), three cases of eccrine adenocarcinoma, two cases of mucinous carcinoma of the skin, six cases of extramammary Paget's disease, a case of mammary Paget's disease, two cases of Merkel cell carcinoma, and two cases of cutaneous metastasis from gastric carcinoma. Each specimen was frozen in liquid nitrogen immediately after biopsy and stored at -70℃ until use. Unfixed frozen sections were made in a cryostat at -30℃ and examined by an indirect immunofluorescence method. The sections were incubated with an antikeratin monoclonal antibody for 30 min at room temperature, washed in

phosphate-buffered saline, and then incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody (Cappel Laboratories, West Chester, USA) for 30 min at room temperature. After washing in phosphate-buffered saline, the sections were mounted in glycerin buffer and observed under a fluorescence microscope (Zeiss Standard 18FL).

The antikeratin monoclonal antibodies used were HKN-2, HKN-4, HKN-5, HKN-6, HKN-7, RKSE60, CK7, LE41, RGE53, A53-B/A2, and LP2K. HKN-2, HKN-4, HKN-5, HKN-6 and HKN-7 were made in our laboratory; their characteristics have been previously reported.[7-9] Briefly, HKN-4 is an antikeratin monoclonal antibody that recognizes a broad spectrum of keratins distributed in both stratified and simple epithelia. HKN-2 is an antikeratin monoclonal antibody that recognizes stratified epithelial keratins. It shows a relatively broad distribution in normal human skin. HKN-5, HKN-6 and HKN-7 show restricted reactions confined to hair tissue in normal skin. RKSE60 and RGE53 were purchased commercially (Bioscience Products, Emmenbrucke, Switzerland), as were CK7, LE41 and LP2K (Ammersham International, Amersham UK), and A53-B/A2 (Progen Biotechnik, Heidelberg, FRG). RKSE60 recognizes stratified epithelial keratin 10, considered to be a marker of epidermal terminal differentiation.[10] The reaction is confined to the epidermis, sebaceous gland, and duct of sweat gland. CK7,[11] LE41[12] and RGE53[13] are specific monoclonal antibodies for simple epithelial keratins 7, 8 and 18, respectively. They stain the secretory cells of sweat gland in normal human skin. Merkel cells are also recognized with LE41 and RGE53, but not with CK7. LP2K[12] and A53-B/A2[14] are monoclonal antibodies that recognize simple epithelial keratin 19. Their reactions are confined to sweat gland, hair follicle and Merkel cell in normal human skin. The reactivities of the antikeratin monoclonal antibodies with normal human skin tissues are summarized in Table 1.

RESULTS

Two cases of Merkel cell carcinoma (Fig. 1A) showed positive reactions with LE41 and RGE53 (Fig. 1B), whereas no reaction was observed with CK7, LP2K, HKN-2, HKN-5 or RKSE60. All the specimens obtained from six patients with extramammary Paget's disease (Fig. 2A) and a patient with mammary Paget's disease showed the same results. CK7, LE41, RGE53 (Fig. 2B), LP2K and A53-B/A2 stained Paget cells located in the epidermis, while the surrounding epidermal keratinocytes were negative to these antibodies. In contrast, the Paget cells were not stained with HKN-2, HKN-5 and RKSE60, although the surrounding epidermal keratinocytes were stained with these antibodies. In two cases of mucinous carcinoma of the skin (Fig. 3A), the tumor cells reacted with LE41, RGE53 (Fig. 3B) and A53-B/

Table 1. Reactivity of Antibodies with Normal Human Skin

	Antikeratin Monoclonal Antibodies							
	HKN-4	HKN-2	RKSE60	HKN-5	HKN-6, HKN-7	A53-B/A2, LP2K	RGE53, LE41	CK7
Epidermis	+	+	+	−	−	−	−	−
Pilar app.								
ORS	+	+	−	+*	−	+§	−	−
IRS	+	+	−	+	+	−	−	−
Hair shaft	+	+	−	+	+	−	−	−
Sebaceous g.	+	+	+	−	−	−	−	−
Sweat gland								
Duct	+	+	+	−	−	+‡	−	−
Myoepithel	+	+	−	−	−	−	−	−
Sec. cell	+	−	−	−	−	+	+	+
Merkel cell	+	−	−	−	−	+	+	−

+, reacting −, not reacting +*, mainly reacting to the innermost cells +§, mainly reacting to the basal cells +‡, reacting to the luminal cells Pilar app., pilar apparatus ORS, outer root sheath IRS, inner root sheath Sebaceous g., sebaceous gland Myoepithel, myoepithelial cell Sec. cell, secretory cell

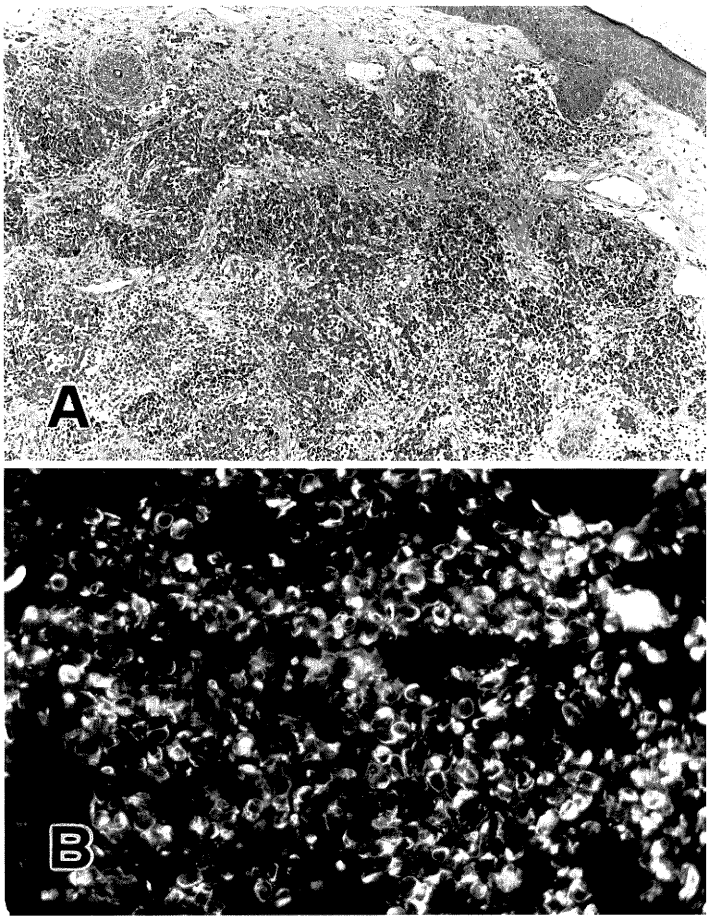

Fig. 1. Merkel cell carcinoma. A. Light microscopic finding. Monomorphous tumor cells with a uniform appearance proliferate in the dermis (hematoxylin-eosin, ×70). B. Fluorescein isothiocyanate immunohistochemical staining with RGE53. Most of the tumor cells are positively stained (×170).

A2, while CK7, HKN-2, HKN-5 and RKSE60 showed no reactions. Two cases of cutaneous metastasis from gastric carcinoma (Fig. 4A) showed positive reactions with LE41, RGE53 (Fig. 4B) and A53-B/A2, while CK7, HKN-2, HKN-5 and RKSE60 revealed no stainings.

In all cases of BCE (Fig. 5A), Bowen's disease, SCC (Fig. 6A), and eccrine adenocarcinoma, most of the tumor cells were positively stained with HKN-2 (Fig. 5B, 6B), while CK7, LE41 and RGE53 showed no reactivities except for a case of eccrine adenocarcinoma. In eleven of the twelve cases of BCE (Fig. 5C), three of the fifteen cases of Bowen's disease, four of the sixteen cases of SCC, and one of the three cases of eccrine adenocarcinoma, positive reactions with A53-B/A2 were observed in varying degrees. The four positive cases of SCC were carcinomas arising in Bowen's disease, but the other twelve negative SCC cases for this antibody were not related to Bowen's disease. In almost all tumor cells with Bowen's disease, a diffuse staining by RKSE60 was found, while in SCC (Fig. 6C) the reactivity of RKSE60 was irregularly observed mainly in the keratinizing tumor cells. In contrast, no reactivity with RKSE60 was present in BCE except for one case which had some keratinizing foci. Scattered tumor cells positively reacted with RKSE60 in two of the three cases of eccrine adenocarcinoma. In twelve of the sixteen cases of SCC, all cases of BCE, and two of the three cases of eccrine adenocarcinoma, HKN-5 showed positive reactivities. In BCE (Fig. 5D), the HKN-5-positive tumor cells were mainly located in the centers of tumor nests. In SCC (Fig. 6D), the reactivity of HKN-5 was mainly found in the keratinizing tumor cells, and the locations of the HKN-5-positive tumor cells were not always considered to

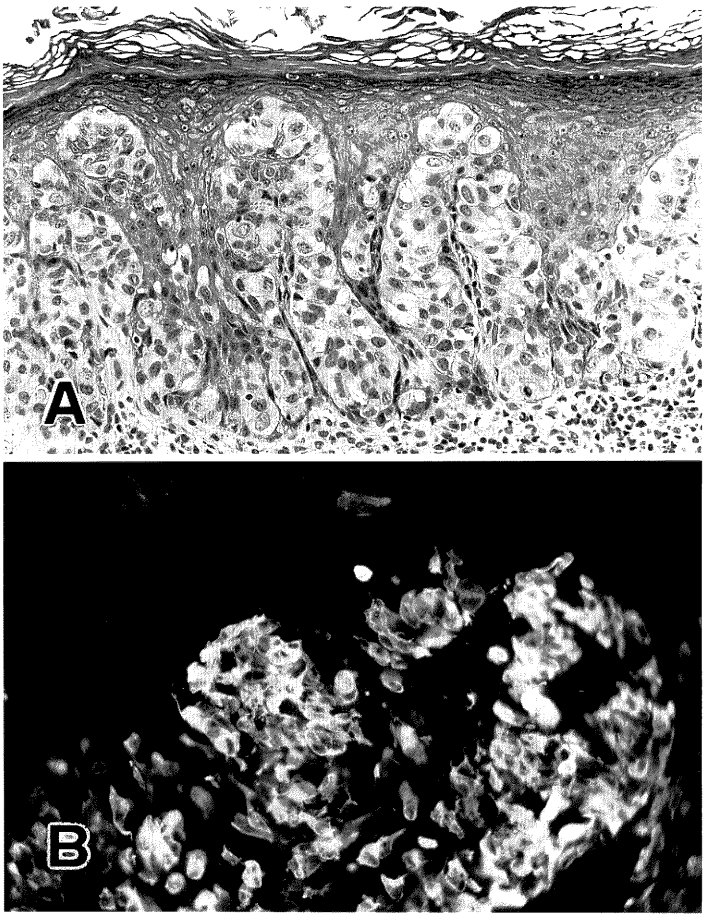

Fig. 2. Extramammary Paget's disease. A. Light microscopic finding. Clusters of Paget cells are present in the epidermis (hematoxylin-eosin, ×170). B. Fluorescein isothiocyanate immunohistochemical staining with RGE53. Clusters of positive Paget cells are present in the epidermis (×170).

correspond to those of RKSE60-positive ones. HKN-4 showed diffusely positive reactions in all cases examined, while HKN-6 and HKN-7 revealed no positive reactions. The reactivities of the antikeratin monoclonal antibodies with carcinomas of the skin are summarized in Table 2.

DISCUSSION

From the present results, the keratin expressions in carcinomas of the skin seem to imitate those in corresponding epithelia in normal human skin according to their differentiations, although the contents of keratins of the carcinomas are not always the same as those in the normal epithelia. Similar to previous biochemical surveys,[15] the tumor cells of Merkel cell carcinoma[16,17] expressed only simple epithelial keratins 8 and 18 as do normal human Merkel cells,[15,18,19] although a simple epithelial keratin 19 is present in the latter[15] but not in the former. Furthermore, neurofilaments are often coexpressed with keratins in Merkel cell carcinomas,[20,21] whereas they are not expressed in normal Merkel cells.[18,19] On the other hand, Paget cells in extramammary Paget's disease expressed only simple epithelial keratins 7, 8, 18 and 19. This finding corresponds to that for the secretory cells of sweat glands in normal human skin.[17,22] It suggests a derivation of Paget cells of extramammary Paget's disease from the secretory cells[23,24] as well as the Paget cells in mammary Paget's disease,[25,26] which are considered to derive from adenocarcinoma arising in mammary gland.[1] Mucinous carcinoma of the skin, considered a subtype of sweat gland carcinomas,[1] also expressed only simple epithelial keratins 8, 18 and 19, but not

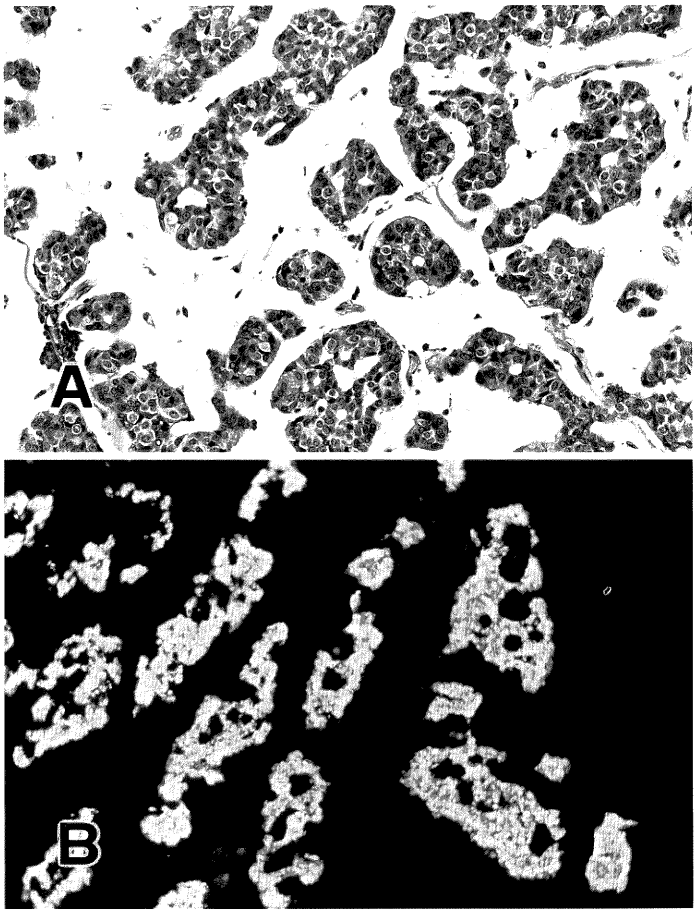

Fig. 3. Mucinous carcinoma of the skin. A. Light microscopic finding. Abundant amounts of mucin surround nests of tumor cells that show lumens (hematoxylin-eosin, ×170). B. Fluorescein isothiocyanate immunohistochemical staining with RGE53. The tumor cells are uniformly stained (×170).

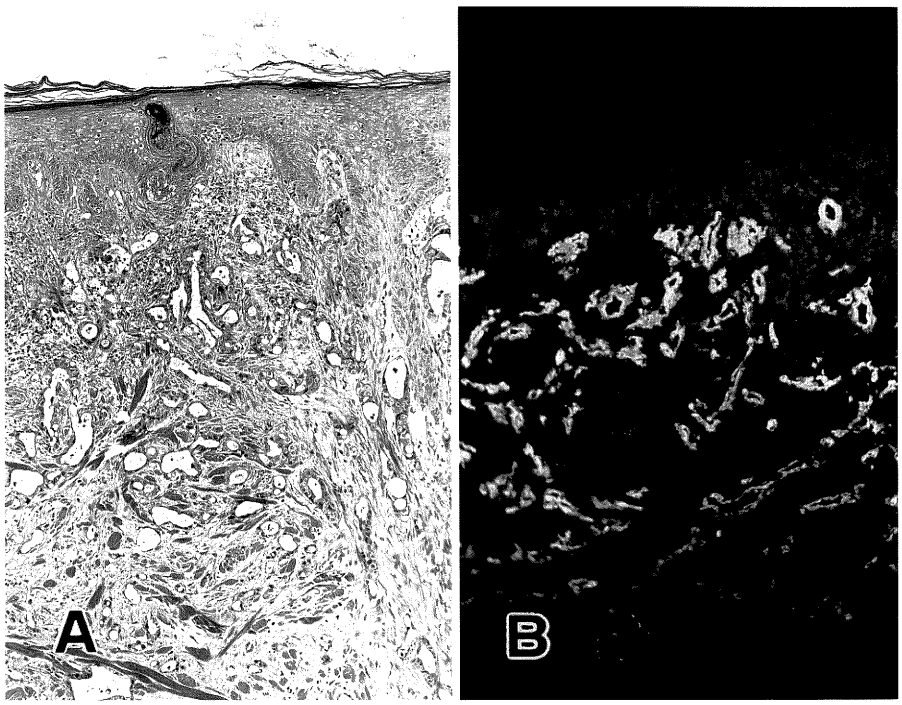

Fig. 4. Cutaneous metastasis from gastric carcinoma. A. Light microscopic finding. Several glandular lumina are present in the dermis (hematoxylin-eosin, ×70). B. Fluorescein isothiocyanate immunohistochemical staining with RGE53. The tumor cells are uniformly stained, while the epidermis shows no positive staining (×70).

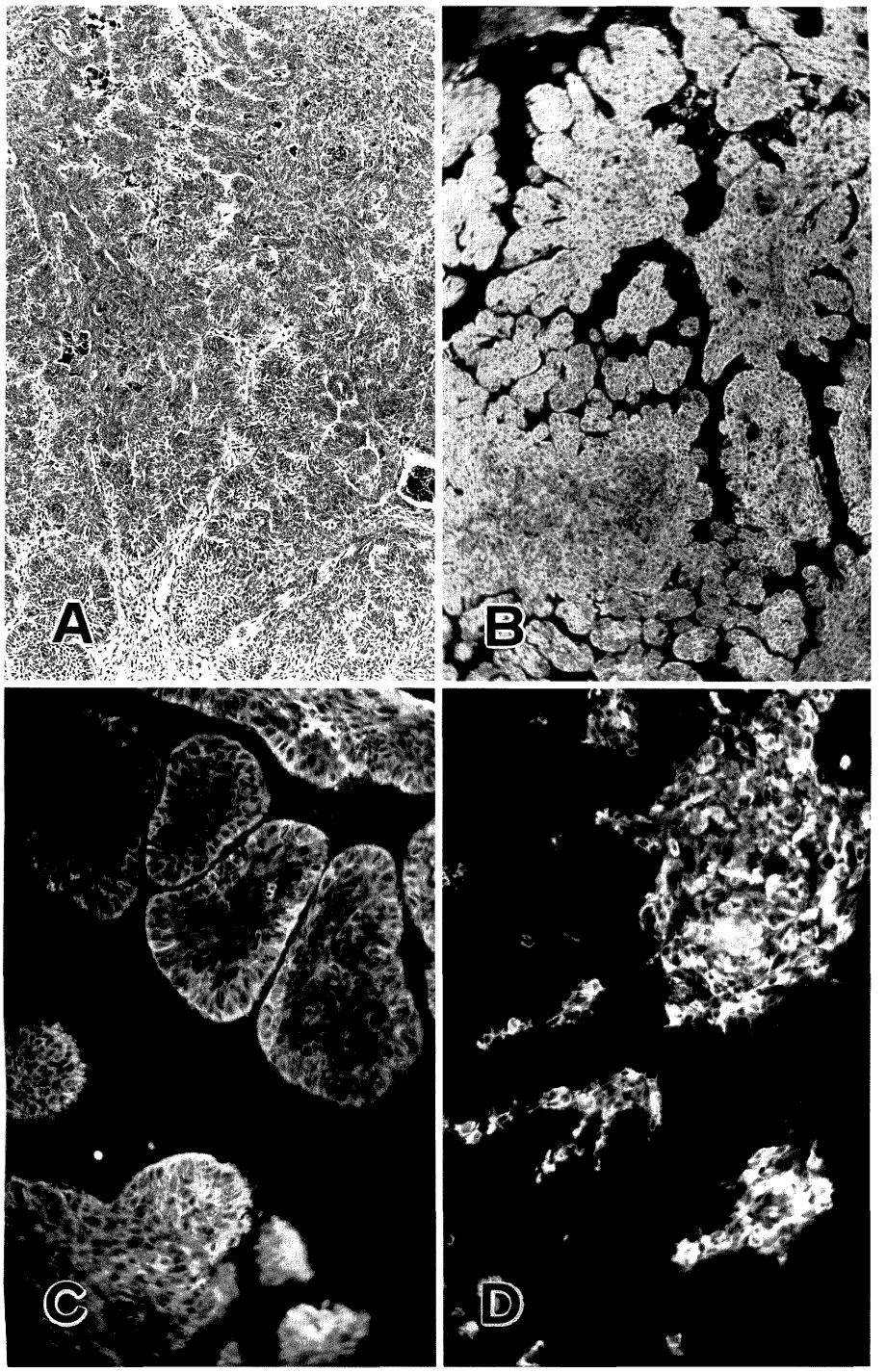

Fig. 5. Basal cell epithelioma. A. Light microscopic finding. Nests of tumor cells proliferate in the dermis (hematoxylin-eosin, ×70). B-D. Fluorescein isothiocyanate immunohistochemical staining (B, HKN-2, ×70. C, A53-B/A2, ×170. D, HKN-5, ×170).

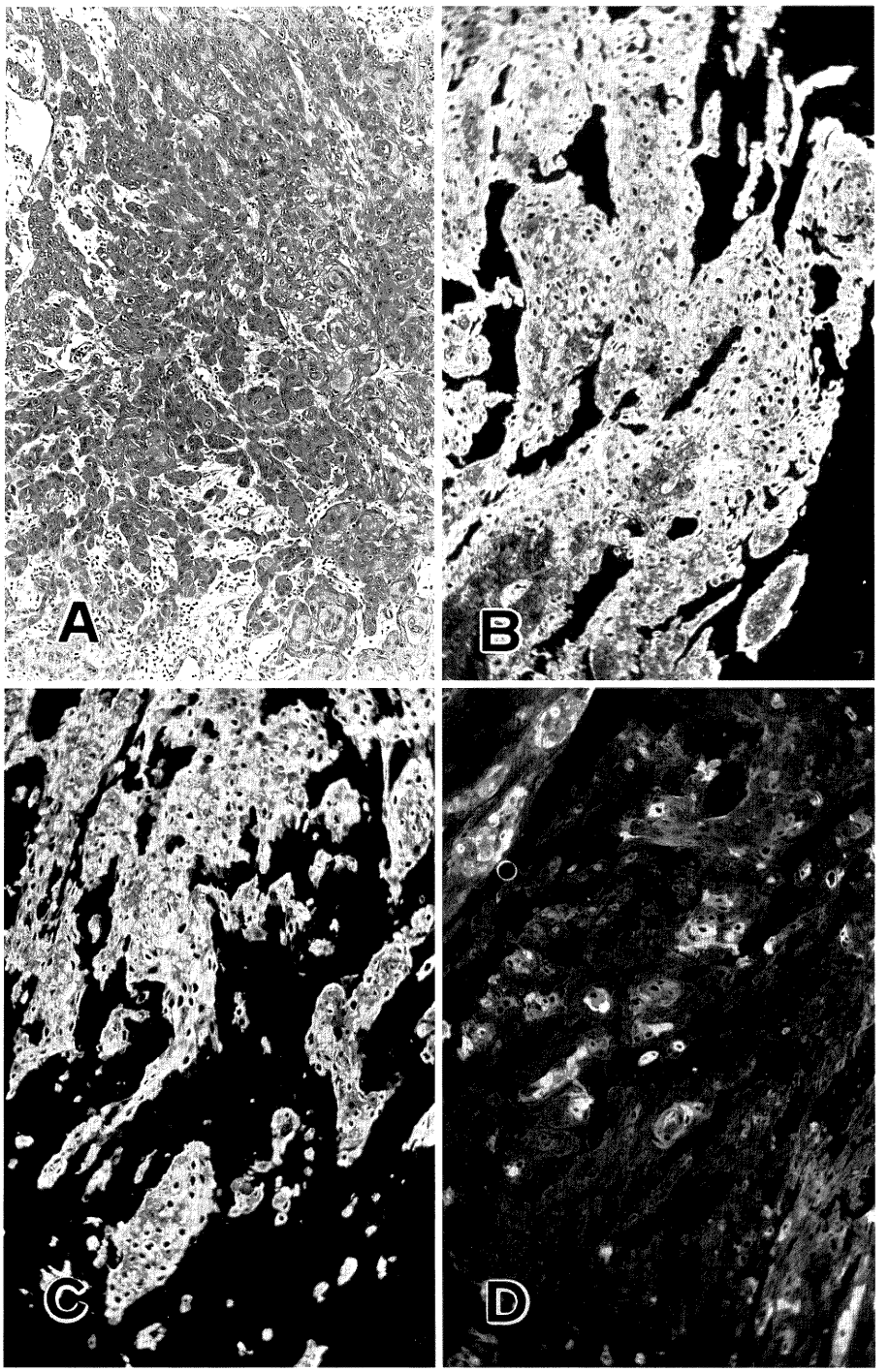

Fig. 6. Squamous cell carcinoma. A. Light microscopic finding. Anaplastic tumor cells proliferate in the dermis (hematoxylin-eosin, ×70). B-D. Fluorescein isothiocynate immunohistochemical staining (B, HKN-2, ×70. C, RKSE60, ×70. D, HKN-5, ×70).

Table 2. Reactivity of Antibodies with Carcinomas of the Skin

	Antikeratin Monoclonal Antibodies							
	HKN-4	HKN-2	RKSE60	HKN-5	HKN-6, HKN-7	A53-B/A2, LP2K	RGE53, LE41	CK7
SCC	+	+	+*	+*	−	−	−	−
SCC (B)	+	+	+*	−§	−	+*	−	−
Bowen	+	+	+	−§	−	−§	−	−
BCE	+	+	−§	+*	−	+*	−	−
EAC (1)	+	+	+*	−§	−	−	−	−
(2)	+	+*	−	−	−	+	+*	+*
MC	+	−	−	−	−	+	+	−
Paget	+	−	−	−	−	+	+	+
Merkel	+	−	−	−	−	−	+	−
GC	+	−	−	−	−	+	+	−

SCC, squamous cell carcinoma SCC (B), carcinoma arising in Bowen's disease Bowen, Bowen's disease
BCE, basal cell epithelioma EAC, eccrine adenocarcinoma MC, mucinous carcinoma of the skin
Paget, Paget's disease Merkel, Merkel cell carcinoma GC, cutaneous metastasis from gastric carcinoma
+, reacting −, not reacting +*, partially reacting −§, exceptionally reacting

keratin 7. The differential diagnosis of the mucinous carcinoma of the skin from cutaneous metastasis from gastric carcinoma seems to be impossible by analyses of keratins.

The simple epithelial keratin 19 was also demonstrated with stratified epithelial keratins recognized with HKN-2, not only in eccrine adenocarcinoma but also in some cases of SCC, Bowen's disease and BCE. In normal human skin, the expression of keratin 19 is confined to adnexal epithelia such as sweat gland and hair tissue.[27] It may be one of markers for such adnexal differentiations. In one case of eccrine adenocarcinoma that histologically revealed well-differentiated glandular structures, the keratin 19 was broadly expressed as well as HKN-2-reacting keratins in most of the tumor cells. On the other hand, simple epithelial keratins 7, 8 and 18 were seen only in the luminal tumor cells. In this case, the former seems to indicate an adnexal differentiation, while the latter may reveal that there is a differentiation toward secretory cells. However, it should be noted that keratin 19 is not always expressed in all cases of eccrine adenocarcinoma, as it was not detected in two cases of eccrine adenocarcinoma in spite of the presence of luminal structures.

BCEs are considered by some dermatologists to be the least differentiated benign appendage tumors —and not carcinomas—because they are composed of immature cells rather than of anaplastic cells.[1] The demonstration of keratin 19 in many tumor cells in most cases of BCE appears to give evidence of the adnexal differentiation. Furthermore, the existence of hair tissue keratins recognized with HKN-5 may support the pilar differentiation of the BCE cells, even in the solid and adenoid types of BCE.[28] However, the differentiations toward hair cortical cells did not seem to be present, because of the negative stainings with HKN-6 and HKN-7 that stain tumors with a hair cortical differentiation.[28–30] In contrast, the stratified epithelial keratin 10, which is a marker for epidermal differentiation, was not detected in most cases of BCE, except for a partial positivity in a keratotic type of BCE. These results may support the previous immunohistochemical and biochemical investigations[5,31,32] proving that the keratin expressions of BCE resemble those of normal hair follicles.

Bowen's disease is thought to be an intraepidermal type of SCC.[1] In some cases of Bowen's disease, certain tumor cells infiltrated into the dermis beyond the epidermal basement membrane, forming an invasive SCC. The expression of keratin 19 in such carcinomas may suggest an adnexal differentiation of the tumor cells. This seems to be supported by the fact that the number of epidermal keratin 10-positive cells decreased in carcinomas arising in Bowen's disease compared with that in Bowen's disease. Histologically, carcinomas arising in Bowen's disease have been reported to have various differentiations.[33] However, apparent differentiations toward neither sweat gland nor pilar apparatus were histologically

observed, and neither simple epithelial keratins 7, 8 and 18 nor hair follicle keratins were expressed in the carcinomas. In contrast, Bowen's disease always expressed keratins similar to those in the normal epidermis, except for a few cases that expressed keratin 19 in some tumor cells.

The tumor cells of SCC that proliferated and infiltrated downward into the dermis expressed only stratified epithelial keratins but none of the simple epithelial keratins 7, 8, 18 and 19. Although the stratified epithelial keratins recognized by HKN-2 were expressed in most tumor cells, the anaplastic tumor cells did not express keratin 10, which is a marker for an epidermal differentiation, except in some keratinizing foci such as horn pearls. In contrast, hair follicle keratins recognized by HKN-5 were detected in some tumor cells, although no cases of SCC expressed keratin 19 that is also present in some epithelial cells in normal hair follicles. Such hair follicle-associated keratins have been shown to be expressed in SCC by biochemical studies using two-dimensional gel electrophoresis.[6]

It is suggested that immunohistochemical examinations may be of great value for the differential diagnosis of carcinomas of the skin, because the carcinomas express individual combinations of keratins according to their differentiations as revealed in the present study.

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