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The Detection of Basal Cell Determinants in Human Basal Cell Carcinomas Using Two Different Monoclonal Antibodies

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This report deals with the reaction pattern(s) of two monoclonal antibodies (MoAbs) with normal skin and basal cell carcinomas (BCC). Using indirect immunoperoxidase (IIP) and indirect immunofluorescence (IIF) techniques, MoAb 12 G7 was observed to react with a determinant related to the cell membrane of the epidermal basal cells. In the IIP technique MoAb 12 G7 showed a positive reaction with 32 out of 34 BCC (94%), while in IIF all the 14 BCC that were studied were positive. In most cases only the cells at the periphery of the tumour nests were stained. MoAb 253 B7 reacted with cytoplasmic determinant(s) of the epidermal basal cells both in the IIF as well as in the IIP techniques. Using the IIP technique only 5 out of 34 BCC (15%) showed a positive reaction with this MoAb. Four of the 5 positively staining tumours showed aggressive histological features. Using IIF technique only 2 out of 14 BCC were positive. The results presented in this communication are discussed with regard to the possible expression of selective differentiation and tumor-associated determinant(s) in BCC. (Received March 19, 1987.)

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Basal cell carcinoma (BCC) is the most common cancer of epithelial origin although metastasis is fortunately extremely seldom (15, 23, 30). However, a proportion of BCC depending on the specific histologic features are aggressive in their behaviour: local destruction leading to mutilation and recurrence after surgical excision (11, 15, 26). It is well known that in malignant epithelial skin tumours differentiation antigens, such as pemphigus antigen (3), basal cell layer antigens (2), upper cytoplasmic antigens (33), β_2-microglobulin (7, 10, 14, 29) and involucrin cannot be detected (20, 25). As in other neoplastic diseases, in epithelial skin tumours too, there are some reports on the presence of tumour-associated antigens (TAA) recognized by monoclonal antibodies (MoAbs), which form an additional aid for the detection of residual tumour cells in Mohs' surgery (1, 19, 22). Investigations, into the cytokeratin composition of normal and neoplastic epithelium have provided insight into the pathogenesis (16, 17, 18, 31, 32). The exclusive expression of cytokeratin 17 in BCC and pilosebaceous tract and not in the normal interfollicular epidermis has been reported previously by Moll et al. (17). This would suggest that the origin of BCC is related to pilosebaceous cells. However, the expression of keratin 17 in cultured normal skin, conjunctival and esophageal keratinocytes, and in hyperproliferative epidermal diseases such as psoriasis have all been previously reported (4, 16, 27, 31). Therefore as stated by Weiss et al. (31), the expression of keratin 17 is not cell-type specific and its presence in BCC should not be taken as evidence for a pilosebaceous origin of the tumour.

The lack of insight into the pathogenesis of BCC, the spectrum of various clinical and histological appearances of BCC (15, 30) and the diagnostic problems associated with some histological types of BCC (13, 15) formed the basis of the current investigations. Recently we reported on the reaction patterns of MoAbs 12 G7 and 253 B7 in normal and psoriatic skin (28). In the present study these two MoAbs were used for investigation into basal cell determinant(s) of BCC.

MATERIALS AND METHODS

Preparation of skin samples

Thirty-four specimens of basal cell carcinomas (BCC) and surrounding normal skin were obtained by surgical excision from 29 patients aged 35 to 88 years. The diagnosis of the tumours were confirmed by examination of haematoxylin and eosin (H & E)-stained frozen sections and stained paraffin embedded sections. The BCC were classified in the conventional manner as described previously (12, 15). For the histopathological examination of the skin adjacent to the tumour, the samples were cut in their length in two equal parts. One portion and the edges of the other portion were fixed in formalin and paraffin embedded for histological confirmation of diagnosis and for determination of tumour free margins. The remaining portion was frozen in liquid nitrogen cooled isopentane and stored in liquid nitrogen. Cryostat sections (5 µm in thickness) were placed on alcohol-cleaned glass slides, air dried and fixed in acetone for 10 min at room temperature. Sections were air-dried and stained immediately for the indirect immunoperoxydase (IIP) procedure or for the indirect immunofluorescence (IIF) procedure. Normal skin samples were obtained from 10 healthy volunteers and were processed in the same way.

Monoclonal antibodies

MoAb 12 G7 was generated after three intraperitoneal immunizations of BALB/C mice with 1×10^7 cells from primary culture of human mesothelioma. Spleen cells from immunized mice were hybridized with SP2/0 cells using the somatic hybridization techniques (9). The hybridoma supernatants were prescreened using the standard ELISA technique and cultured mesothelioma cells. They were also prescreened on cryostat skin sections using IIF technique. MoAb 253 B7 was obtained after three intraperitoneal immunizations of BALB/C mice with an extract of total tumour material of human skin squamous cell carcinoma, prepared using an ultra-Turrax homogenizer. Spleen cells from immunized mice were hybridized with P_3 myeloma cells (9). The hybridoma supernatants were prescreened on cryostat sections of normal skin and squamous cell carcinoma using IIP technique. MoAb 253 B7 was subcloned twice and 10^7 cells were injected intraperitoneally in BALB/C mice. After 10 days the ascites fluid was collected. A titer of 1 : 1200 was used in the IIP and IIF techniques.

The results of prescreening showed that the 2 MoAbs reacted with the basal cell layer of normal epidermis.

The 2 MoAbs were produced at the Dept. of Cell Biology and Genetics.

Indirect immunofluorescence test (IIF)

The staining pattern of the epidermis adjacent to the tumour and the epidermis at a distance (>150 µm) was studied in 14 BCC. Dry fixed cryostat sections were preincubated for 30 min with a 1:20 dilution of bovine serum albumin (BSA) to reduce aspecific staining. The sections were then incubated for 60 min at room temperature with one of the MoAbs, rinsed with phosphate buffered saline (PBS, pH 7.4) and incubated for 30 min with Fluorescein isothiocyanate (FITC) labelled rabbit-antimouse IgG (Dakopatt, Copenhagen, Denmark) at a dilution of 1:50.

The sections were then rinsed with PBS and mounted in glycerol-PBS (9: 1) solution. A Leitz Ortholux fluorescence microscope equipped with filters for epi-illumination and narrow band excitation was used for examining the sections.

Indirect immunoperoxidase test (IIP)

The cryostat sections were preincubated with BSA at a dilution of 1 : 20 for 30 min. The sections were then incubated with one of the MoAbs for 60 min, rinsed in PBS and incubated with rabbit peroxidase-conjugated antimouse IgG at a dilution of 1:50. The peroxidase reaction was developed by incubating the sections with 3,3′-diaminobenzidine (DAB) at a concentration of 0.5 mg/ml and hydrogen peroxide (0.01 %) for several minutes at room temperature. Sections were then rinsed in tap water, counter-stained with haematoxylin for 1–2 min and rinsed again in tap water. The sections were mounted in Malinol (Chroma-Gesellschaft, Stuttgart). All sera were diluted in PBS, pH 7.4.

Fig. 1. Normal skin cryostat section (5 μm) MoAb 12 G7, IIP technique. ×340. The staining is confined to the membrane region of the epidermal basal cells.

In both procedures the controls comprised: antikeratin MoAbs K80 (Sanbio b.v., Uden, The Netherlands) and K92 (Dakopatts, Holland) were used as positive controls (21, 24) and negative controls were performed by omitting either the primary antibody or the rabbit-antimouse immunoglobulin.

RESULTS

Normal skin

In the 10 normal skin samples MoAb 12 G7 reacted consistently with membrane related determinant(s) of epidermal basal cells as observed in the IIF and IIP (Fig. 1). MoAb 12 G7 also showed a positive reaction with the outer root sheath of the hair follicles, sebaceous glands and the ducts and the secretory portions of the sweat glands. MoAb 253 B7 showed consistently a diffuse staining of the cytoplasm of the epidermal basal cells (Fig. 2) and

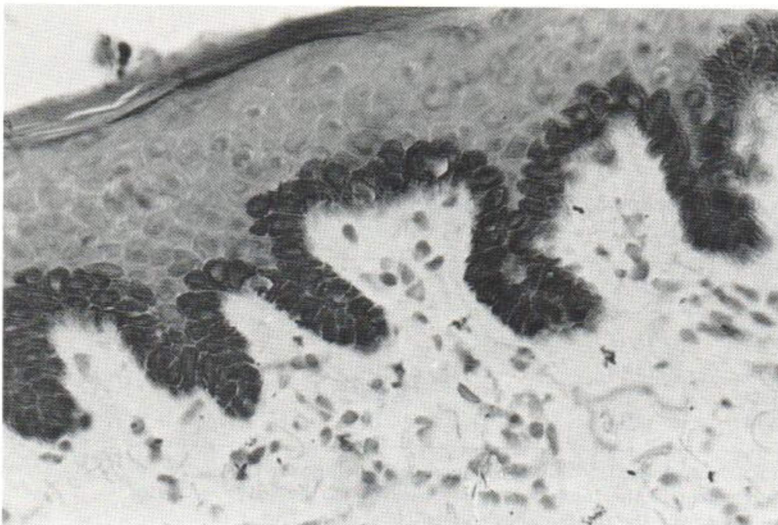

Fig. 2. Normal skin cryostat section (5 μm) MoAb 253 B7, IIP technique. ×340. The staining is restricted to the cytoplasm of the epidermal basal cell layer.

also stained the outer root sheath of the hair follicles, sebaceous glands, sweat glands, especially the ducts and the myoepithelial cells of the secretory ducts, smooth muscle fibers of blood vessels and musculi arrector pili.

Basal cell carcinomas (BCC)

Thirty-four BCC from 29 patients were evaluated. Twenty-eight were located in head and neck region, four were in the trunk region and two were located on one of the extremities. The histological subclassification of BCC is summarized in Table I.

Table II shows a summary of the reactivity pattern of MoAbs 12 G7 and 253 B7.

MoAb 12 G7: in the IIP, 32 out of 34 BCC (94%) stained positively. The peripheral cell layers of most tumour nests stained strongly, whereas the center of the tumour nest stained either very weakly or none at all (Figs 3 *a* and 3 *b*). In the IIF all 14 BCC evaluated were positive.

MoAb 253 B7: only 5 out of 34 BCC (15%) stained positively in the IIP. All tumour cells in the tumour nests and strands of tumour cells were stained, except the tumour cells with squamous-like differentiation in the keratotic BCC and in the basosquamous BCC (Fig. 4). The other 29 BCC stained either very weakly or none at all. The 5 positively staining tumours comprised 2 morphoeic type, 1 keratotic type, 1 fibrosing infiltrated type and 1 basosquamous type. Both in the IIF and in the IIP no diminishing staining was observed adjacent to the tumour. The IIF staining pattern is shown in Fig. 5.

DISCUSSION

The results of this report show that MoAb 12 G7 and 253 B7 reacted with different determinants of the epidermal basal cells. MoAb 12 G7 probably reacted with a membrane

Table I. *Histological subclassification of 34 basal cell carcinomas from 29 patients*

Histological type	Number of tumours
Solid	16
Keratotic	2
Cystic	2
Adenoid	2
Infiltrating	5
Morphoeic	3
Superficial	1
Pigmented	1
Basosquamous	2

Table II. *Reactivity patterns of monoclonal antibodies (MoAbs) 12 G7 and 253 B7 in normal skin and basal cell carcinomas*

MoAb	NE	Tumour	EPA	EPD	Staining technique
12 G7	10/10	32/34	34/34	34/34	IIP
	10/10	14/14	14/14	14/14	IIF
253 B7	10/10	5/34	34/34	34/34	IIP
	10/10	2/14	13/14	14/14	IIF

NE = no. of normal skin biopsies which showed positively staining basal cell layer, EPA = no. of tumours in which the epidermis adjacent to the tumour showed positive staining, EPD = no. of tumours in which the epidermis distant from the tumour showed positive staining.

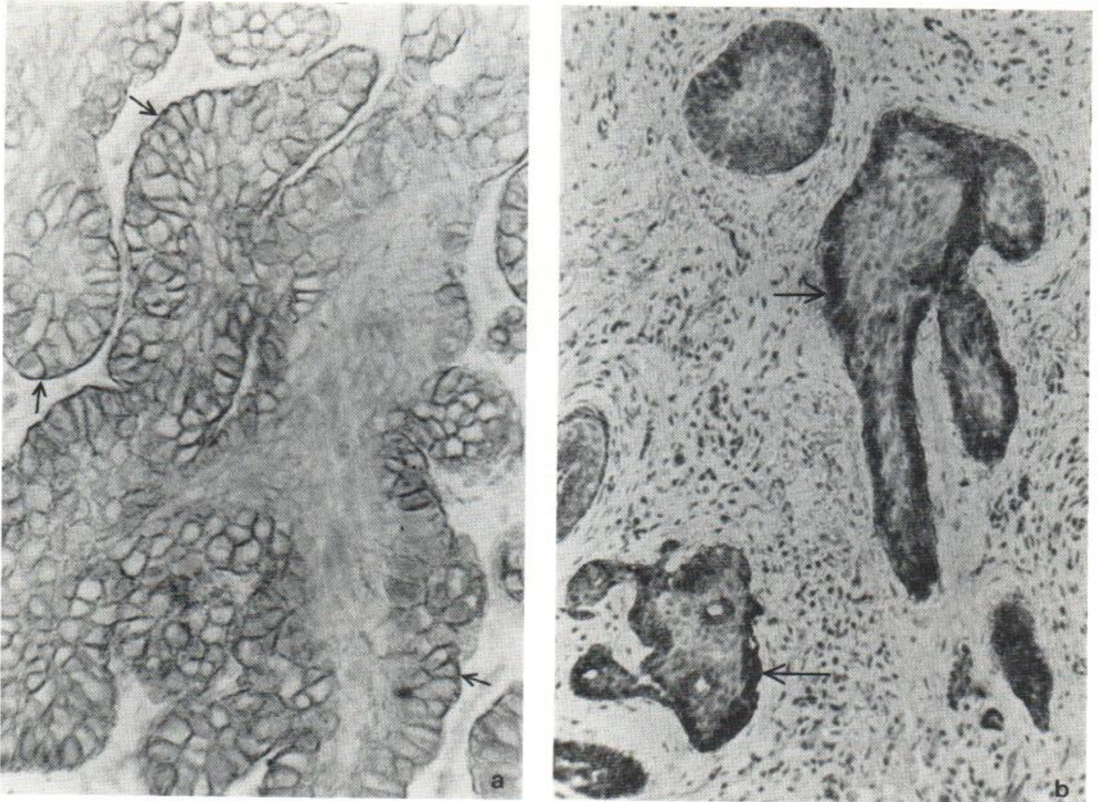

Fig. 3 a and *3 b*. Cryostat sections (5 μm) of solid type BCC and infiltrating type BCC respectively, MoAb 12 G7, IIP technique. ×340 and ×135, respectively. Tumour cells at the periphery of the tumour nests are predominantly stained (arrows).

related determinant and MoAb 253 B7 reacted with a cytoplasm determinant. Both MoAbs did not react exclusively with skin epithelium; 12 G7 reacted with mesothelium and 253 B7 with smooth muscle cells and myoepithelial cells. In the current studies we observed that MoAb 12 G7 reacted positively in 32 out of 34 BCC (94%) and MoAb 253 B7 reacted positively only in 5 out of 34 BCC (15%). There are several reports in the recent literature concerning MoAbs VM-1 (22), VM-2 (19) and PKK-2 (8), which react with normal epidermal basal cells and BCC. Eto et al. (5) described MoAb EKH4 which reacted with the lower 2–3 cell layers of the normal epidermis and upon immunoblotting was found to react predominantly with a 50 KD keratin. MoAb EKH4 also reacted positively with BCC. Each of the above mentioned MoAbs reacted consistently with all the tumour nests of BCC. However, in the present study, using MoAb 12 G7 only the cells at the periphery of the tumour nests were strongly stained, whereas the cells at the center of the tumour nests were either stained very weakly or none at all. In most cases, the tumour cells in the center of the tumour nests showed on light microscopical level aspects of differentiation (elongated cells with an oval pale nucleus). The differentiated tumour cells in keratotic BCC and basosquamous BCC also did not react with MoAb 12 G7. A possible explanation for this observation might be that the tumour cells, which show some degree of differentiation, may have lost this determinant(s) as compared to that observed in the suprabasal cellayer of normal epidermis. Another explanation may be that the tumour cells at the center of the

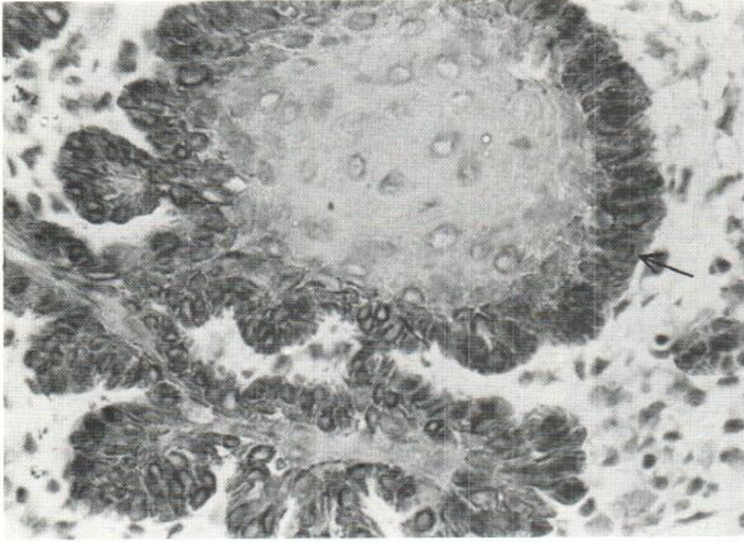

Fig. 4. Cryostat section (5 µm) of basosquamous BCC, MoAb 253 B7, IIP technique. ×340. Tumour cells at the periphery stain strongly (arrow) whereas squamous-like cells in the center of the tumour nests remain unstained.

tumour nests become less vital and lose some markers due to physiologic factors, such as diminished availability of nutrients. In this respect the findings recently reported by Grimwood et al. (5) are of particular interest. They observed that dividing cells in nodular BCC were mostly located at the periphery of tumour nests. The cells in the center of the

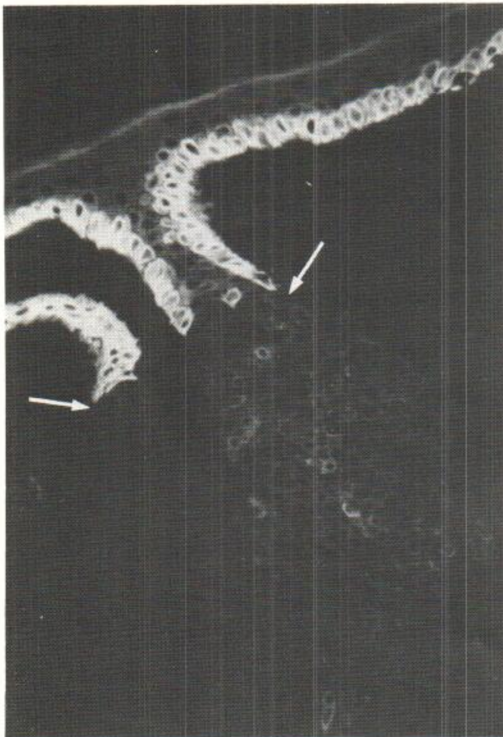

Fig. 5. Cryostat section (5 µm) of solid type BCC, MoAb 253 B7, IIF technique. ×340. The epidermis nearby the tumour showed a strong staining with an abrupt change to no staining of the adjacent tumour cells (arrows).

tumour nests had lost their capacity to divide and had undergone some type of differentiation similar to normal epithelial cells.

MoAb 253 B7 reacted only with 5 out of 34 BCC (15%). Conspicuously 4 out of 5 positively staining tumours were of aggressive type. The other 29 out of 34 BCC stained either very weakly or not at all. Probably the cytoplasmic determinant is not lost but there is a variation in the quantity of the expression of this determinant in BCC. Whether the quantity of expression of this determinant is a marker for the aggressive behaviour is subject to speculation, especially, since 6 out of 10 tumors with aggressive histological features did not stain positively. The exact nature of the determinants detected by the two MoAbs 12 G7 and 253 B7 is currently under investigation. However, our present results lead to the conclusion that the MoAbs 12 G7 and 253 B7 detect different determinants in basal epidermal cells and tumour cells of BCC. Therefore, these two MoAbs may be helpful in elucidating the pathogenesis of basal cell carcinomas.

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