

p63 Constitutes a Useful Histochemical Marker for Differentiation of Pagetoid Bowen's Disease from Extramammary Paget's Disease

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Sir,

Pagetoid Bowen's disease (PBD) is a histological variant of Bowen's disease that contains cells morphologically resembling those of extramammary Paget's disease (EPD). In both PBD and EPD, the atypical cells display an abundant, pale-staining cytoplasm in a pagetoid distribution within the epidermis. Although it is relatively easy to diagnose PBD histopathologically in many cases, there are some in which it is difficult to make a definite histopathological differentiation from EPD. In such cases, immunohistochemical studies facilitate differentiation. In general, the neoplastic cells in PBD are negative for cytokeratin (CK) 7, carcinoembryonic antigen (CEA) and gross cystic disease fluid protein 15 (GCDFF-15), whereas the neoplastic cells (Paget's cells) in EPD are positive for them (1, 2). However, CK7 and CEA have been reported also to be expressed in some cases of PBD (3).

p63 is a member of the p53 gene family. It is essential for regulating the proliferation and differentiation of squamous cells, as well as oncogenesis (4, 5). In the skin, p63 is expressed in the epidermis and sebaceous glands, but not in the secretory cells of the apocrine and eccrine glands (6). These characteristics of p63 led us to evaluate its usefulness as a marker to differentiate PBD, a squamous carcinoma *in situ*, from EPD, an adenocarcinoma *in situ*.

MATERIALS AND METHODS

We collected archival formalin-fixed paraffin-embedded lesional specimens from 5 patients with PBD and 5 with EPD, which were kept at the Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan. Over the preceding 10-year period (1993–2007), there were 5 cases of PBD. The

specimens were examined with a mouse anti-p63 monoclonal antibody (4A4, Neomarkers, Fremont, CA, USA) and mouse anti-CEA antibody (Takara, Tokyo, Japan). Immunohistochemical staining was performed on 4 µm specimens. For p63 staining only, the slides were immersed in 0.01 M citrate buffer (pH 6.0) and autoclaved at 121°C for 5 min. The slides were blocked with serum and then incubated overnight at 4°C with the respective primary antibodies. Antibody binding was demonstrated using a biotin-streptavidin-peroxidase method (Histofine SAB-PO kits, Nichirei, Tokyo, Japan) and visualized with 3,3'-diaminobenzidine tetrahydrochloride (Wako Pure Chemicals, Osaka, Japan).

RESULTS

The cases of PBD (age range 63–83 years) consisted of 2 males and 3 females in whom the lesions developed on the extremities, flank, or face. In contrast, EPD occurred in the inguinal region in 4 males and 1 female (age range 54–75 years). The clinical pictures of both diseases showed a slowly enlarging reddish patch with a sharp, irregular border.

In PBD, the salient microscopic features were the presence of neoplastic cells showing abundant pale-staining cytoplasm and atypical nuclei (Fig. 1a). They tended to form well- to poorly-defined cell groups or existed individually from the basal layer to the granular layers. Immunohistochemical staining with p63 showed strongly positive reactivity in the nuclei of the neoplastic cells in all of the 5 cases of PBD (Fig. 1b). The intensity of p63 in the large neoplastic cells was similar to that noted in normal keratinocytes, which served as a useful internal control. In addition, CEA immunohistochemical staining displayed negative reactivity in all cells (Fig. 1c).

In EPD, there were atypically large cells with abundant, pale-staining cytoplasm (Fig. 2a). Their

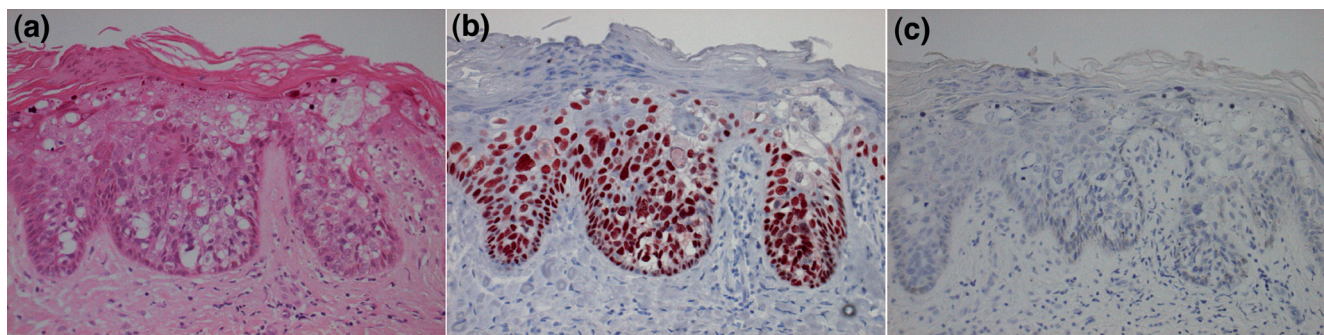


Fig. 1. Pagetoid Bowen's disease. (a) The presence of large palely stained cells within the epidermis (haematoxylin-eosin, ×100). (b) p63 immunohistochemical staining (×100). The neoplastic cells are positive for p63. The staining of normal basal keratinocytes serves as an internal control. (c) Negative carcinoembryonic antigen (CEA) immunohistochemical staining (×100). (b, c: nuclei were counterstained with haematoxylin.)

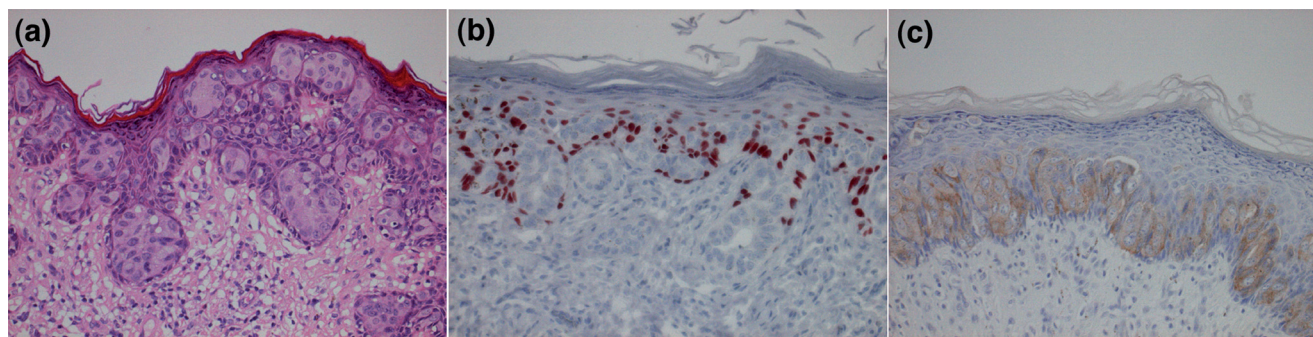


Fig. 2. Extramammary Paget's disease. (a) Large palely stained cells in the epidermis (haematoxylin-eosin, $\times 100$). (b) p63 immunohistochemical staining ($\times 100$). The Paget's cells are negative for p63, whereas the normal keratinocytes are positive for it. (c) Positive carcinoembryonic antigen (CEA) immunohistochemical staining ($\times 100$). (b, c: nuclei were counterstained with haematoxylin.)

histopathological findings resembled those of PBD. However, these Paget's cells were negative for p63, in sharp contrast to the surrounding keratinocytes (Fig. 2b). Moreover, they showed clear expression of CEA in all cases (Fig. 2c).

DISCUSSION

We have demonstrated the presence of p63 in the neoplastic cells of PBD, in contrast to its total absence in the Paget's cells of EPD.

The pagetoid pattern of PBD is noted only as a histopathological feature of the epidermal involvement. It is unlikely that such a transition to the pagetoid form is accompanied by any difference in biological behaviour. However, diagnostic problems in examining their histology sometimes occur (7). Currently, numerous antibody preparations are available for a differential diagnosis. The neoplastic cells of PBD are negative for CEA, CK7, CAM5.2 and GCDFP-15, in contrast to Paget's cells, which are positive for them (1, 2). However, a few cases of EPD were reported to be not stainable with CEA or CK7 (8). Moreover, PBD is occasionally positive for CEA and CK7, making it difficult to make a definite differentiation (3). To enhance the sensitivity and specificity of our diagnostic kit, several antibodies, rather than a single one, should be employed.

p63 plays important roles in the control of epithelial tissues, especially that of the stratified epithelia (4, 5). p63 knockout mice suffer from severe defects in the stratified epithelia (5). Moreover, the importance of p63 in the epithelium is exemplified by the fact that Hay-Wells syndrome, a p63 gene mutation syndrome, is characterized by denuded skin and scalp erosion, which seem to occur from abnormalities of keratinocyte stem cells (9). In the skin, p63 is expressed in the basal and spinous cells of the epidermis, germinative cells of sebaceous glands, and myoepithelial cells of the sweat glands. On the other hand, secretory cells of the sweat glands do not express p63 (6). Based on the p63 expression in the stratified epithelia, it is reasonable to

conclude that p63 is positive in PBD but not in EPD. However, p63 was positive in a minor population of subjects with adenocarcinoma and melanoma (10). Further studies in a large group are necessary to clarify the sensitivity and specificity of p63 as the diagnostic marker.

The specific expression of p63 in PBD indicates that p63 constitutes a useful marker for its histopathological diagnosis. We propose to add p63 to the repertoire to make a definite differentiation between PBD and EPD.

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