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Mucin immunohistochemistry in the diagnosis and mapping of extramammary Paget's disease

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Abstract

Extramammary Paget's disease (EMPD) is a rare skin cancer of the genital region in which cancer cells with enlarged nuclei and pale cytoplasm are scattered singly in the affected epidermis. These cancer cells, called Paget cells, contain mucin, which is never found in normal epidermis. The oligosaccharide side chains of Paget cell mucin end with sialic acid. Sialic acid is easily detected by zirconyl haematoxylin or alcian blue. The other sugars in the oligosaccharide chains can be detected by the periodic acid-Shiff reaction. Rarely, the diagnosis of EMPD is complicated by the absence of mucin from the Paget cells. We have examined such an atypical case. The oligosaccharide side chains, including the sialic acids, are absent. In both this case and a typical case, the Paget cells contain epithelial membrane antigen mucin (MUC1) core protein and usually contain gastric surface-type mucin (MUC5AC) core protein, which can be stained by antibodies. Since neither core protein is found in normal epidermis, epithelial membrane antigen core protein may be the most reliable diagnostic marker for extramammary Paget's disease. In both the atypical case and the typical case of Paget's disease, some cells that look like keratinocytes contain mucin core proteins. These may be incipient Paget cells. We suggest that using the epithelial membrane antigen core protein as a marker for the true extent of extramammary Paget's disease could facilitate complete excision and reduce the rate of recurrence.

Keywords: apomucin • epithelial membrane antigen • extramammary Paget's disease • mucin • mucin core protein • MUC1 • MUC5AC • Paget cells

Introduction

Extramammary Paget's disease (EMPD) is a rare epidermal carcinoma that most often appears in the anogenital region [1]. It resembles Paget's disease of the nipple in appearing as isolated Paget cells or small groups of Paget cells rather than as a continuous mass [2, 3].

Typical Paget cell morphology includes a large nucleus and pale cytoplasm. Paget cells usually contain sialomucins [4, 5]. The presence of sialomucin is one way of distinguishing malignant Paget cells from benign Toker cells [6] and from the malignant cells of Bowen's disease [1, 7, 8]. All three cell types appear as groups of 1–50 large cells with enlarged nuclei and pale cytoplasm in H&E or trichrome preparations; they can be confused if the diagnosis is made on the basis of morphology alone. An immunohistochemical re-evaluation of morphological diagnoses of extramammary Paget's disease, Bowen's disease and superficial spreading malignant melanoma found a 5% error rate in the original diagnoses [9]. The risks of such a mistake are serious: Toker cells are a common benign anomaly [6, 10, 11], and Bowen's disease can usually be treated with topical chemotherapy alone [12, 13], but EMPD usually requires surgery [14, 15] or prolonged radiotherapy [16].

Sialomucins are easily stained with zirconyl haematoxylin or alcian blue. All mucins are stained by the periodic acid Schiff (PAS) reaction.

The occasional absence of mucin in EMPD has led to the suggestion that mucin staining should be supplemented by at least one immunohistochemical stain in all cases of suspected

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EMPD [17, 18]. The presence of cytokeratin 7 usually distinguishes EMPD from Bowen's disease [19, 20], but not from Toker cells [21].

The recent availability of antibodies to human mucin core proteins has led to a search for specific mucin markers to distinguish EMPD from similar skin lesions and to determine the extent of EMPD. Mucous neck cell-type mucin (MUC6) has never been found in Paget cells [5, 22, 23]. Intestinal type mucin (MUC2) has only rarely been found in Paget cells [5, 23]. Gastric surface-type mucin (MUC5AC) is often found in EMPD [5, 22, 23].

Epithelial membrane antigen (EMA), also known as episialin or MUC1, has the chemical structure of a mucin, but it is normally a transmembrane glycoprotein rather than a secreted glycoprotein [24, 25, 26]. Paget cells usually contain sialylated intracellular EMA in both extramammary and mammary Paget's disease [1]. EMA is absent from Toker cells [10, 11]. EMA is weakly expressed in Bowen's disease, and it is usually confined to the cell membrane [27, 28]. Unlike the sialylated EMA usually found in EMPD, the EMA found in Bowen's disease usually has little or no sialic acid and does not stain with Alcian blue [1].

Rarely, the diagnosis of extramammary Paget's disease is complicated by the absence of sialomucins from the Paget cells [17]. Finding a case of non-mucin-secreting EMPD led us to ask if the mucin core proteins might be present without their oligosaccharide side chains.

Materials and methods

This protocol was approved by Barry University's Institutional Review Board .

Slides of formalin-fixed paraffin-embedded sections of two cases of EMPD of the vulva were obtained from the Co-operative Human Tissue Network.

Several patients undergoing cosmetic surgery donated their tissues, which served as normal controls. Pieces of labia minora from two patients, pieces of perineal skin from two female patients and a fragment of skin from the medial thigh from one male patient were fixed in 10% formalin, embedded in paraffin, and cut at 7 μm. A slide from each case was stained with Ehrlich's haematoxylin and eosin Y [29], zirconyl haematoxylinand methylene green [30], alcian blue at pH 2.5 and kernechtrot [31] and the PAS reaction [32].

Two sections from each case of EMPD were de-paraffinized, heated to 95°C for 40 min. in 0.10 M pH 6.0 citrate buffer to retrieve the antigen, treated with 0.3% hydrogen peroxide in methanol for 30 min. to quench endogenous peroxidase activity, followed by 1.5% normal horse serum (Vector) in PBS for 20 min., incubated 6 hr at 23°C in a 1:200 dilution of mouse monoclonal antibodies (clone MAB 2011, Chemicon, Temecula, CA) to MUC5AC in 1.5% normal horse serum, washed in PBS, incubated 30 min in 0.5% biotinylated horse anti-mouse immunoglobulin (Vector) in PBS, washed in PBS, treated with avidin-conjugated horseradish peroxidase (Vector ABC kit) for 30 min. and washed in PBS. One section was stained with Vector's Nova Red for 5 min. and counterstained in haematoxylin; the second section was stained with Vector's VIP for 2 min. and counterstained in kernechtrot [31]. One section from each of the five normal control tissues was incubated in the same way and stained with Nova Red. All sections were dehydrated, cleared and mounted in Permount (Fisher, Atlanta, GA). A control section from each case of EMPD was treated similarly with the omission of the mouse antibodies to MUC5AC and stained with Nova Red.

The mouse antibodies to MUC5AC were raised against a synthetic polypeptide of the consensus tandem repeat of human MUC5AC core protein: threonine-threonine-serine-threonine-threonine-serine-alanine-proline [33, 34, 35].

One section from each case was de-paraffinized, blocked with 0.3% hydrogen peroxide in methanol for 30 min. followed by 1.5% normal horse serum (Vector, Burlingame, CA) in PBS for 20 min., incubated 30 min. at 23°C in a 1:100 dilution of mouse monoclonal antibodies (clone ZCE 113, Zymed, San Francisco, CA) to human EMA in 1.5 % normal horse serum (Vector), washed in PBS, incubated 30 min. in 0.5 % biotinylated horse antimouse immunoglobulin (Vector), treated with avidin-conjugated horseradish peroxidase (Vector ABC kit) for 30 min., stained with Vector's Nova Red for 15 min., counterstained in haematoxylin, dehydrated, cleared and mounted in permount. One section from each of the five normal control tissues was treated the same way. Another section from each case of EMPD was treated similarly with the omission of the mouse antibodies to EMA.

The mouse antibodies to EMA were raised against cream from human milk, which contained membrane-bounded fat globules. Thus, the antibodies were raised against the glycosylated EMA.

Results

Both cases of EMPD showed many cells with typical Paget cell morphology (enlarged cells with an enlarged nucleus and pale cytoplasm) in the epidermis of sections stained with haematoxylinand eosin The Paget cells in slides from the typical case stained with zirconyl haematoxylin (Fig. 1), strongly with the PAS reaction, and brilliantly with alcian blue. The Paget cells in slides from the other case did not stain at all with zirconyl haematoxylin (Fig. 2) or alcian blue, establishing the absence of sialomucins. They did not stain with the PAS reaction, showing the absence of any kind of mucin. Normal keratinocytes in both cases of EMPD and in the control tissues did not stain with zirconyl haematoxylin or alcian blue, but they did stain faintly with PAS.

Many Paget cells in the typical sialomucin-positive (Fig. 3) and the atypical sialomucin-negative (Fig. 4) case reacted with monoclonal antibody to MUC5AC mucin core polypeptide. In both cases, a few cells that did not have a Paget cell morphology reacted with antibody to MUC5AC polypeptide. Controls slides of EMPD with the antibody omitted did not stain.

Live keratinocytes in the control tissues never stained with antibody to MUC5AC, but light background staining was often seen in the stratum corneum (Fig. 5). Rarely, a few sebaceous glands stained faintly.

Almost all Paget cells in both cases reacted strongly with monoclonal antibody to EMA (Figs. 6–7). In both cases, a few cells that did not have Paget cell morphology reacted with antibody to EMA. Control slides of Paget's disease with the antibody omitted did not stain.

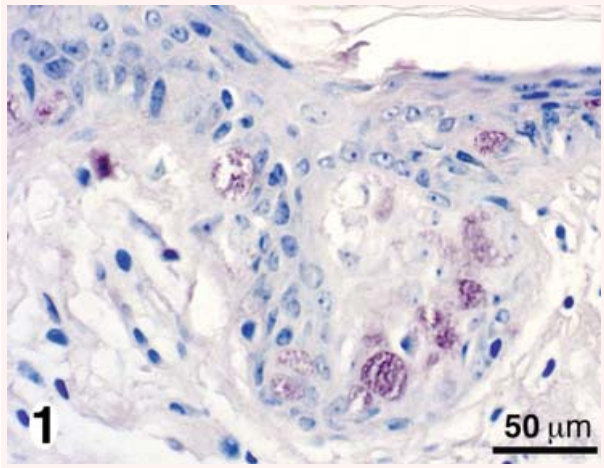

Fig. 1 Sialomucin in typical Paget cells stains purple with zirconyl haematoxylin. The nuclei stain with methylene green.

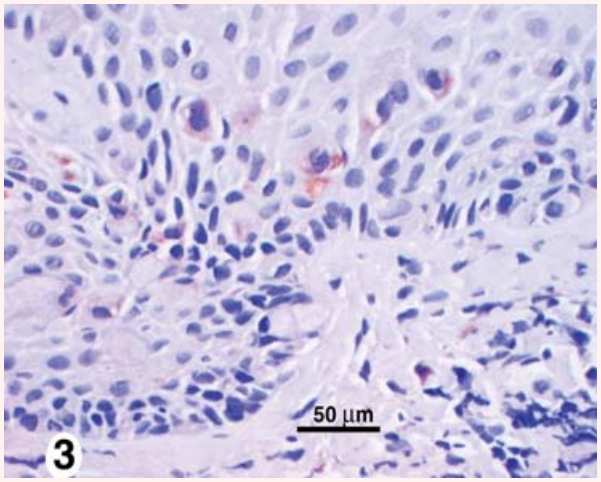

Fig. 3 Paget cells in mucin-positive extramammary Paget's disease contain MUC5AC core protein (red–brown reaction product).

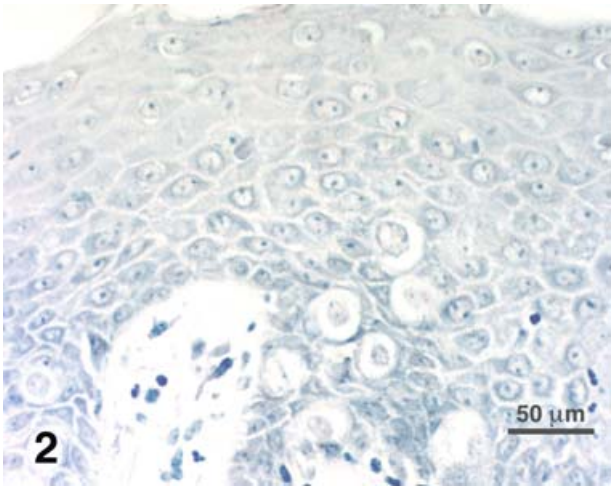

Fig. 2 Paget cells in mucin-negative extramammary Paget's disease fail to stain with zirconyl haematoxylin. The nuclei and rough ER stain with methylene green.

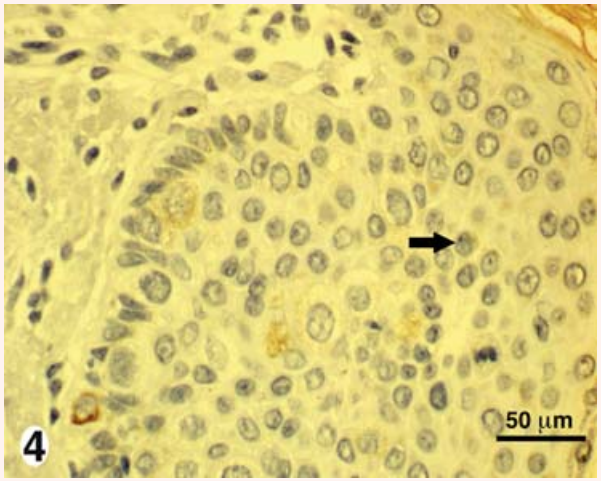

Fig. 4 Many Paget cells in mucin-negative extramammary Paget's disease contain MUC5AC core protein (red–brown reaction product). A morphologically normal cell (arrow) also contains MUC5AC core protein (red–brown reaction product).

No cells in the control skin from the thigh or from the perineum reacted with antibody to EMA. Sebaceous glands in both control labia minora reacted strongly with antibody to EMA; keratinocytes did not react (Fig. 8). Even the epithelium around a microscopic condyloma in one labium minus did not react with antibodies to EMA. (The patient was referred to her gynaecologist for treatment of the underlying human papilloma virus infection.)

Discussion

It is common knowledge that normal epidermis does not contain sialomucins [1, 4, 5]. Where normal skin has been used as a control, neither MUC5AC and EMA core proteins have been noticed in the epidermis [23]. This study searched for MUC5AC and EMA core proteins in normal epidermis and found neither.

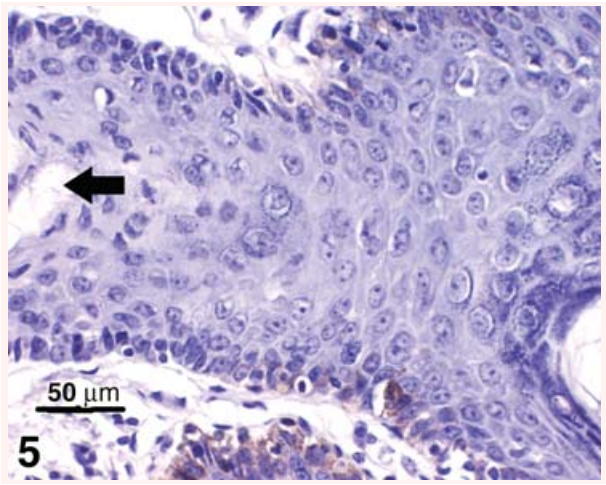

Fig. 5 No cells in a control labium minus stain for MUC5AC core protein. Melanin in the stratum basale is grey–brown unlike the red–brown of Nova Red. The sebaceous gland (arrow) is unstained.

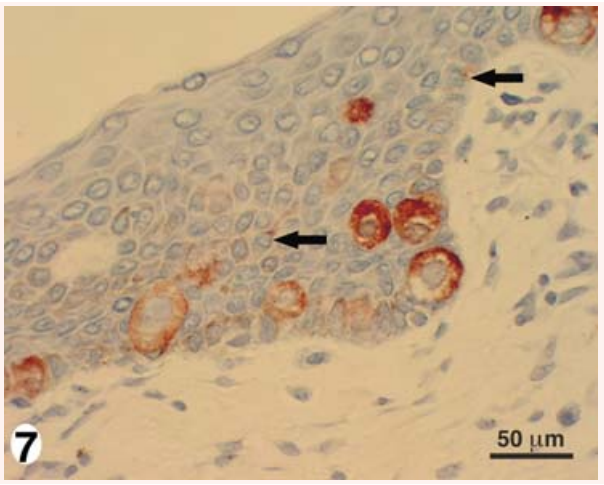

Fig. 7 Mucin-negative extramammary Paget's disease. The Paget cells and two morphologically normal cells (arrows) contain epithelial membrane antigen (red–brown reaction product).

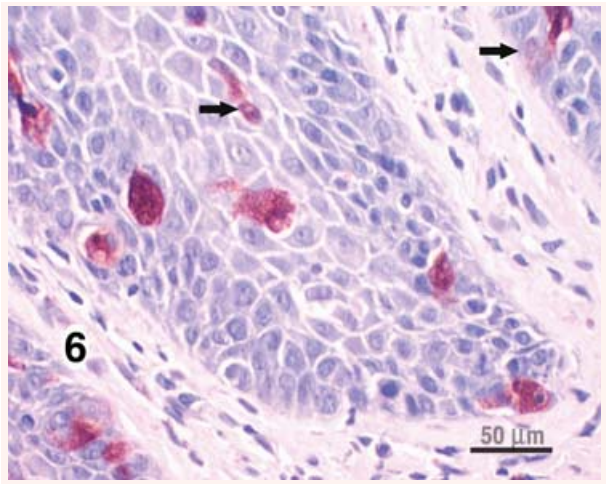

Fig. 6 Mucin-positive extramammary Paget's disease. The Paget cells and two morphologically normal cells (arrows) contain epithelial membrane antigen (red–brown reaction product).

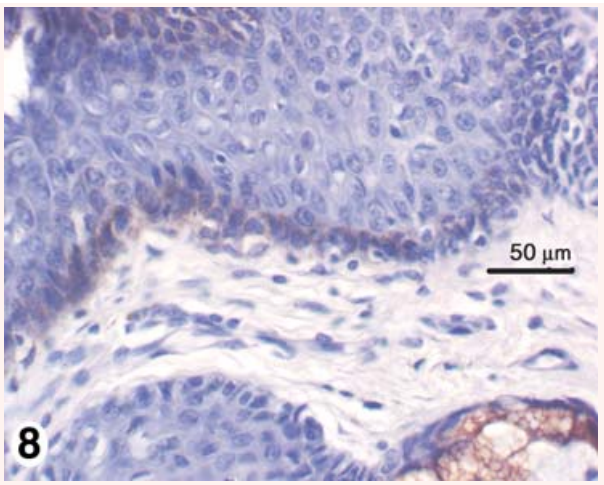

Fig. 8 Normal labium minus incubated with antibody to epithelial membrane antigen. Note the difference between the red–brown stain in the sebaceous gland and the grey–brown melanin in the stratum basale.

The staining of normal keratinocytes with PAS is presumably due the presence of glycogen. It is notable that the non-mucin-secreting Paget cells contained less glycogen than the surrounding normal keratinocytes.

We started our staining of MUC5AC core protein by following Yoshii et al. [22]. Our antibody (from Chemicon) and their antibody (from Novocastra) were both monoclonal antibodies to synthetic polypeptides with the same amino acid sequence. Nevertheless, our antibody bound more quickly and less specifically than theirs, forcing us to use a shorter incubation time to eliminate background staining. Liegl *et al.* [23], using twice our antibody concentration, found that their antibody (from Eubio) bound to Paget cells in less than half their cases. The wide variation in the experience of different research groups suggests that

MUC5AC core protein is not a reliable marker for the diagnosis of extramammary Paget's disease. The failure of some Paget cells in our cases to bind MUC5AC antibody also suggests that MUC5AC core protein would not be a reliable marker for mapping the extent of EMPD.

The staining of mucin-negative Paget cells with antibodies to EMA, that is MUC1, raised against human milk fat globules confirms previous observations [36, 37] that many antibodies generated against the complete glycoprotein bind to the core protein. It is believed that the most antigenic portion of the complete glycoprotein is the 20 amino acid tandem repeat polypeptide [38].

(It has been suggested that Paget cells may be malignantly transformed Toker cells [10, 11]. The appearance of antigens typical of Paget cells in cells that resemble keratinocytes rather than Toker cells, suggests that Paget cells arise from keratinocytes rather than Toker cells.)

Although neither sialic acid residues nor the distribution pattern of EMA core protein are infallible markers for the diagnosis of EMPD, each is useful when used separately and, when used together, greatly enhance the accuracy of diagnosis. The almost universal presence of EMA in Paget cells (5, 22) makes it a good marker for determining the extent of the disease. It would be especially useful for scouting biopsies [39] and Mohs surgery.

The presence of EMA and MUC5AC core protein in a few cells that did not have the morphology of Paget cells suggests the possibility that they may be incipient Paget cells. If so, extramammary Paget's disease arises from many cells and removal of all cells with Paget cell morphology often leaves some incipient Paget cells behind. This would account for the high rate of recurrences after surgical excision of EMPD [40–42]. If our surmise is correct, mapping the margins of extramammary Paget's disease with antibodies to EMA should reduce the rate of recurrence after surgical excision.

(By detecting incipient Paget cells before they attain Paget cell morphology, immunohistochemical detection of epithelial membrane antigen could make surgical margins more accurate and sharply reduce the rate of recurrence.)

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References

1. **Lloyd J, Flanagan AM.** Mammary and extramammary Paget's disease. *J Clin Pathol.* 2000; 53: 742–9.
2. **Eller JJ, Eller WD.** Tumors of the Skin. Philadelphia: *Lea & Febiger;* 1951.
3. **Jones RE, Austin C, Ackerman AB.** Extramammary Paget's disease: a critical reexamination. *Am J Dermatopathol.* 1979; 1: 101–32.
4. **Sasai Y, Nakama T, Kasuda M.** Sialomucin in Paget cells of extrammary Paget's disease. *Histochem J.* 1983; 15: 987–97.
5. **Kondo Y, Kashima K, Daa T, Fujiwara S, Nakayama I, Yokoyama Y.** The ectopic expression of gastric mucin in extramammary and mammary Paget's disease. *Am J Surg Pathol.* 2002; 26: 617–23.
6. **Toker C.** Clear cells of the nipple epidermis. *Cancer.* 1970; 25: 601–10.
7. **Helwig EB, Graham JH.** Anogenital (extramammary) Paget's disease. A clinicopathological study. *Cancer.* 1963; 16: 387–403.
8. **Odom RB, James WD, Berger TG.** Andrews' diseases of the skin. 9th ed. Philadelphia: Saunders; 2000.
9. **Reed W, Oppedal BR, Eeg Larsen T.** Immunohistology is valuable in distinguishing between Paget's disease, Bowen's disease and superficial spreading malignant melanoma. *Histopathology.* 1990; 16: 583–8.
10. **Marucci G, Betts CH, Golouh R, Peterse JL, Forschini MP, Eusebi V.** Toker cells are probably precursors of Paget cell carcinoma: a morphological and ultrasound description. *Virchows Arch.* 2002; 44: 117–23.
11. **Wilman JH, Golitz LE, Fitzpatrick JE.** Vulvar clear cells of Toker: precursor of extramammary Paget's disease. *Am J Dermatopathol.* 2005; 27: 185–8.
12. **Rosen T, Harting M, Gibson M.** Treatment of Bowen's Disease with topical 5% imiquimod cream: retrospective study. *Dermatol Surg.* 2007; 33: 427–32.
13. **Patel MJ, Stockfleth E.** Does progression from actinic keratosis and Bowen's disease end with treatment: diclofenac 3% gel, an old drug in a new environment? *Br J Dermatol.* 2007; 156 Suppl 3: 53–6.
14. **Pierie JP, Choudry U, Muzikansky A, Finkelstein DM, Ott MJ.** Prognosis and management of extramammary Paget's disease and the association with secondary malignancies. *J Am Coll Surg.* 2003; 196: 45–50.
15. **Thomas CJ, Wood GC, Marks VJ.** Mohs micrographic surgery in the treatment of rare aggressive cutaneous tumors: the Geisinger experience. *Dermatol Surg.* 2007; 13: 333–9.
16. **Yanagi T, Kato N, Osawa R.** Radiotherapy for extramammary Paget's disease: histopathological findings after radiotherapy. *Clin Dermatol.* 2007; 32: 506–8.
17. **Alguacil-Garcia A, O'Connor R.** Mucin-negative biopsy in extra-mammary Paget's disease. A diagnostic problem. *Histopathology.* 1989; 15: 429–31.
18. **Kohler S, Rouse RV, Smoller BR.** The differential diagnosis of pagetoid cells in the epidermis. *Mod Pathol.* 1998; 11: 70–92.
19. **Lundquist K, Kohler S, Rouse RV.** Intraepidermal cytokeratin 7 expression is not restricted to Paget cells but is also seen in Toker cells and Merkel cells. *Am J Surg Pathol.* 1999; 23: 212–9.
20. **Shah KD, Tabibzadeh SS, Gerber MA.** Immunohistochemical distinction of Paget's disease from Bowen's disease and superficial spreading melanoma with the use of monoclonal cytokeratin antibodies. *Am J Clin Pathol.* 1987; 88: 689–95.
21. **Willamson JD, Colome M I, Sahin A, Ayala AG, Medeiros J.** Pagetoid Bowen's

disease, a report of 2 cases that express cytokeratin 7. *Arch Pathol Lab Med.* 2000; 124: 427–30.

22. **Yoshii N, Kitajima S, Yonezawa S, Matsukita S, Setoyama, Kanazaki T.** Expression of mucin core proteins in extramammary Paget's disease. *Pathol Int.* 2002; 52: 360–89.
23. **Liegl B, Gogg-Kamerer M, Tessaro E, Horn L-C, Moinfar F.** Mammary and extramammary Paget's disease: an immunohistochemical study of 83 cases. *Histopathology.* 2007; 50: 439–47.
24. **Litvinov SV, Hilkens J.** The epithelial sialomucin, episialin, is sialylated during recycling. *J Biol Chem.* 1993; 268: 21364–71.
25. **Moniaux N, Escande F, Porchet N, Aubert J-P, Batra SK.** Structural organization and classification of the human mucin genes. *Front Biosci.* 2001; 6: D1192–206.
26. **Schofield DP, Simms MS, Bishop MC.** MUC1 mucin in urological malignancy. *BJU Int.* 2003; 91: 560–6.
27. **Tatemoto Y, Saka M, Tanimura T, Mori M.** Immunohistochemical observations on binding of monoclonal antibodies to epithelial membrane antigen in epithelial tumors of the oral cavity and skin. *Oral Surg Oral Med Pathol.* 1987; 64: 721–6.
28. **Cooper HL, Cook IS, Theaker JM, Mallipeddi R, McGrath J, Friedmann, Healy E.** Expression and glycosylation of MUC1 in epidermolysis bullosa-associated and sporadic cutaneous squamous cell carcinomas. *Brit J Dermatol.* 2004; 151: 540–5.
29. **Lillie RD.** Histopathologic Technic and Practical Histochemistry. 2nd ed. *New York, Blakiston,* 1954.
30. **McNulty JM, Kambour MJ, Smith AA.** Use of an improved zirconyl hematoxylin stain in the diagnosis of Barrett's esophagus. *J Cell Mol Med.* 2004; 8: 382–7.
31. **Kiernan JA.** Histological and Histochemical Methods. 3rd ed. Oxford, Butterworth-Heinemann, 2000.
32. **Davenport HA.** Histological and histochemical technics. Philadelphia, Saunders, 1960.
33. **Guyonnet-Duperat V, Audie J-P, Debailleul V, Laine A, Buisine M-P, Galiegue-Zoutina S, Pigny P, Degand P, Aubert J-P, Porchet N.** Characterization of the human mucin gene MUC5AC: a consensus cysteine-rich domain for 11p15 mucin genes? *Biochem J.* 1995; 305: 211–9.
34. **Gendler SJ, Spicer AP.** Epithelial mucin genes. *Ann Rev Physiol.* 1995; 57: 607–34.
35. **Reis CA, David L, Nielsen PA, Clausen H, Migorodskaya K, Roepstorff P, Sobrino-Simoes M.** Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibocy. *Int J Cancer.* 1997; 74: 112–21.
36. **Karanikas V, Patton K, Jamieson G, Pietersz G, McKenzie I.** Affinity of antibodies to MUC1 antigens. *Tumor Biol.* 1997; 19: 71–8.
37. **Pietersz GA, Li W, Osinski C, Apostolopoulos V, McKenzie IF.** Definition of MHC-restricted CTL epitopes from non-variable number of tandem repeat sequences of MUC1. Vaccine 2000; 18: 2059–71.
38. **Karsten U, Serttas N, Paulsen H, Danielczyk A, Goletz S.** Binding patterns of DTR-specific antibodies reveal a glycosylation-conditioned tumor-specific epitope of the epithelial mucin (MUC1). *Glycobiology* 2004; 14: 681–92.
39. **Appert DL, Otley CC, Phillips PK, Roenigk RK.** Role of multiple scouting biopsies before Mohs micrographic surgery for extramammary Paget's disease. *Dermatol Surg.* 2005; 31: 1417–22.
40. **Fanning J, Lambert L, Hale TM, Morris PC, Schuerch C.** Paget's disease of the vulva: prevalence of associated vulvar adenocarcinoma, invasive Paget's disease, and recurrence after surgical excision. *Am J Obstet Gynecol.* 1999; 180: 24–7.
41. **Hendi A, Brodland DG, Zitelli JA.** Extramammary Paget's disease: surgical treatment with Mohs micrographic surgery. *J Am Acad Dermatol.* 2004; 51: 767–73.
42. **Shepherd V, Davidson EJ, Davies-Humphreys J.** Extramammary Paget's disease. *Brit J Obset Gynecol.* 2005; 112: 273–9.