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

R. F. Smith a, B. H. Stern b, A. A. Smith c, *

a School of Nursing, Barry University, Miami Shores, Florida, USA
b Cosmetic Surgery, P.A., Ft. Lauderdale, Florida, USA
c School of Graduate Medical Sciences, Barry University, Miami Shores, Florida, USA

Received: July 26, 2007; Accepted: November 23, 2007

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
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 haematoxylin and methylene green [30], alcian blue at pH 2.5 and kernechtrot [31] and the PAS reaction [32].

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., incubated 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 haematoxylin and 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.
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.
The staining of normal keratinocytes with PAS is presumably due to 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...
that did not have the morphology of Paget cells suggests the
cial useful for scouting biopsies [39] and Mohs surgery.  
EMPD, each is useful when used separately and, when used
grows that Paget cells arise from keratinocytes rather than Toker cells.)  
Although neither sialic acid residues nor the distribution pat-
tern 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 espe-
cially 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

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 con-
firms previous observations [36, 37] that many antibodies gener-
ated against the complete glycoprotein bind to the core protein. It
is believed that the most antigenic portion of the complete glyco-
protein is the 20 amino acid tandem repeat polypeptide [38].

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

Acknowledgements

The authors thank the Co-operative Human Tissue Network for the two
cases of extramammary Paget's disease. The authors also thank the five
patients who consented to the research use of tissue removed during cos-
metic surgery. They thank Barry University School of Graduate Medical
Sciences for providing all of the financial support of the work. The authors
have no financial interest in this work.

References

1. Lloyd JF, 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: Lee & 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 extramammary 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 extramam-
mary and mammary Paget's disease. Am J Surg Pathol. 2002; 26: 617–23.
6. Toker C. Clear cells of the nipple epider-

7. Helwig EB, Graham JH. Anogenital (extra-
mammary) Paget's disease. A clinicopathological study. Cancer. 1963; 16: 407–403.
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 distin-
guishing between Paget's disease, Bowen's disease and superficial spreading malign-
ant melanoma. Histopathology. 1990; 16: 583–8.
10. Marucci G, Bellas CH, Golouh R, Peterse JL, Forschini MP, Eusebi V. Toker cells are probably precursors of Paget cell carci-
noma: a morphological and ultrasound description. Virchows Arch. 2002; 44:
11: 17–23.
11. Wilmam 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, Hartling 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 keratoses and Bowen’s disease end with treatment: diclofenac 3% gel, an
old drug in a new environment? Br J Dermatol. 2007; 156 Suppl 3: S3–6.
14. Pierie JP, Choudry U, Muzikansky A, Finkelstein DM, Ott MJ. Prognosis and Management of Extramammary Paget’s Disease and the Associated with Second-
ary malignancies. J Am Coll Surg. 2003; 196: 45–50.
15. Thomas CJ, Dock GC, Marks VJ. Mohs micrographic surgery in the treatment of rare aggressive cutaneous tumors: the
Geisenger experience. Dermatol Surg. 2007; 13: 333–9.
16. Yanagi T, Kato N, Osawa R. Radiotherapy for extramammary Paget’s disease: histopathological findings after radiothera-
apy. Clin Dermatol. 2007; 32: 506–8.
17. Aigucci-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 diffe-
erential 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.
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. Kieman 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 consensust 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 C, 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 antibody. Int J Cancer. 1997; 74: 112–21.

36. Karanikas V, Patton K, Jamieson G, Pietersz G, McKenzie I. Affinity of anti-bodies 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). Glycobio 2004; 14: 681–92.

39. Apert 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 Obstet Gynecol. 2005; 112: 273–9.