Acta Dermatovenerol Croat 2004;12(4):251-256 ORIGINAL SCIENTIFIC ARTICLE

Cytokeratin 10 and Ki-67 Nuclear Marker Expression in Keratoacanthoma and Squamous Cell Carcinoma

Aleksandra Basta-Juzbašić[1], Sanja Klenkar[1], Jasminka Jakić-Razumović[2], Aida Pašić[1], Davorin Lončarić[1]

[1]Department of Dermatology and Venerology and [2]Department of Pathology, Zagreb University Hospital Center, Zagreb, Croatia

Corresponding author:

Prof. Aleksandra Basta-Juzbašić, MD, PhD

Department of Dermatology and Venerology

Zagreb University Hospital Center

Šalata 4

10000 Zagreb, Croatia

Received: 20.02.2004.
Accepted: 20.10.2004.

SUMMARY The most frequent consideration in the clinical and histologic differential diagnosis of keratoacanthoma is squamous cell carcinoma. In the present study, cytokeratin 10 expression and proliferation rate as measured by Ki-67 expression were compared between 50 clinically and histologically diagnosed keratoacanthomas and 50 squamous cell carcinomas. Tissue sections from the skin were immunohistochemically stained with anti-cytokeratin 10 and anti-Ki-67 monoclonal antibodies. The distribution of cytokeratin 10 expression and proliferative cell count were analyzed. Study results showed higher cytokeratin 10 expression in keratoacanthomas than in squamous cell carcinomas and different distribution of staining in the two entities. The analysis of cytokeratin 10 expression showed a much wider range of values and statistically higher median ($p<0.001$) in keratoacanthomas than in squamous cell carcinomas. Additionally, the proliferation index of keratinocytes as measured by Ki-67 expression was significantly higher in squamous cell carcinomas than in keratoacanthomas ($p<0.01$). These results may prove helpful in histologic differentiation of these disorders.

KEY WORDS cytokeratin 10 and Ki-67 expression; keratoacanthoma; carcinoma, squamous cell; immunohistochemistry

INTRODUCTION

Keratoacanthoma (KA) is a common benign cutaneous tumor that often occurs on sun exposed sites in light-skinned, predominantly middle-aged or older persons (1). Almost all keratoacanthomas arise from hair follicle (2). Occasionally, it is seen on hairless areas such as a nail bed or oral cavity (3,4). It is characterized by rapid growth with a histologic pattern often suggestive of squamous cell carcinoma (SCC) (5). It may be best viewed as an aborted malignancy that only rarely progresses into invasive SCC. SCC is a relatively common skin tumor which, if untreated, generally has a progressive clinical course with development of metastatic disease and often lethal outcome (6). However, KA usually undergoes spontaneous regression as part of its natural history. Clinically, rapid tumor growth may suggest a *de novo* cutaneous SCC, a relatively rare, aggressive tumor that produces regional or distant metastases in about 8% of patients (7). Sometimes a well differentiated SCC can be difficult to distinguish from a KA without clinical history (8). Attempts to distinguish SCC and KA based on the findings of intralesional elastic fibers, intracytoplasmic glycogen, DNA changes and mutant p53 oncogene expression have shown different results but the majority failed to show significant difference (9-14). Recently, it has been shown that there are some differences in p53 and Ki-67 expression be-

Acta Dermatovenerol Croat
2004;12(4):251-256

tween normal skin, psoriatic skin, keratoacanthomas, basal and squamous cell carcinomas (15). Since sometimes it is very difficult to distinguish a histologically well differentiated SCC from KA, in this study we used immunohistochemical method for expression of cytokeratin 10 (CK10) and measurement of cell proliferation rate by Ki-67 in KA and SCC lesions.

MATERIAL AND METHODS

Fifty biopsy specimens of patients with KA and 50 patients with SCC were analyzed. Strict clinical and histologic criteria were used to differentiate SCC from KA. Skin biopsy specimens were fixed in formalin, embedded in paraffin, cut in 3-micrometer sections, and stained with hematoxylin-eosin for routine histologic analysis. Additionally, 4-micrometer sections were cut for immunohistochemical staining with primary mouse monoclonal anti-Ki-67 antibody (clone MIB1, Isotype IgG1, kappa, Dako, Glostrup, Denmark) (1:50 dilution) and anti-CK10 antibody (clone DE-K10, Isotype IgG1, kappa, Dako Glostrup, Denmark) (1:50 dilution) using standard immunoperoxidase streptavidin-avidin method. Briefly, sections were dehydrated in gradient alcohol and then incubated with 3% H_2O_2 for 10 minutes. After that, sections were washed with phosphate buffered saline (PBS), pH 7.4 for 15 minutes, and then incubated with primary antibodies for 30 minutes. Sections were then incubated with conjugate of avidin-biotin peroxidase (Dako) for 30 minutes. After washing in PBS, the reaction was visualized with diaminobenzidine (DAB; Dako) in PBS containing 0.01% H_2O_2 for 5 minutes and counterstained with hematoxylin-eosin. Positive controls were sections of normal skin stained with the same antibodies, whereas negative controls were done by omitting primary antibody. All slides were analyzed by one observer in blind manner using light microscope. In case of sections stained with anti CK10 antibody there were large contiguous areas of tumor cells that were positively stained. Estimating individual cell counts was not possible. The positively stained areas were therefore expressed as percentage of total tumor area on one representative histologic slide using ocular grid. Distribution of CK10 expression was noted as diffuse, focal stain-

Table 2. Results of analysis CK10 and Ki-67 expression and distribution of positive cells in patients with squamous cell carcinomas (SCCs)*

Order	CK 10		Ki67	Order	CK 10		Ki67	Order	CK 10		Ki67
number	%	Distribution	/100	number	%	Distribution	/100	number	%	Distribution	/100
1.	5	2	53	18.	10	6	20	35.	5	2	19
2.	90	5	82	19.	15	6	32	36.	10	2	52
3.	30	3	21	20.	10	6	18	37.	15	6	62
4.	2	2	75	21.	15	6	32	38.	5	2	18
5.	25	6	68	22.	10	6	45	39.	20	6	62
6.	2	2	38	23.	15	6	18	40.	10	2	22
7.	25	6	44	24.	15	6	24	41.	5	2	15
8.	5	2	87	25.	5	2	18	42.	16	6	22
9.	10	6	58	26.	10	2	12	43.	5	2	18
10.	15	6	29	27.	18	2	20	44.	10	6	25
11.	10	6	32	28.	20	6	62	45.	5	6	50
12.	10	6	45	29.	5	2	12	46.	10	6	68
13.	10	6	41	30.	3	6	60	47.	15	6	70
14.	15	6	93	31.	10	2	20	48.	10	6	49
15.	25	6	15	32.	30	6	18	49.	3	2	60
16.	15	6	12	33.	5	2	38	50.	4	6	98
17.	5	2	12	34.	10	6	28				

*1 – suprabasal; 2 – scattered individual tumour cells; 3 – small groups of tumour cells; 4 – suprabasal and small groups of cells; 5 – suprabasal and individual tumour cells; 6 – scattered individual cells and small groups of tumour cells.

Table 1. Results of analysis CK10 and Ki-67 expression and distribution of positive cells in patients with keratoacanthomas (KAs)*

Order	CK 10		Ki67	Order	CK 10		Ki67	Order	CK 10		Ki67
number	%	Distribution	/100	number	%	Distribution	/100	number	%	Distribution	/100
1.	22	1	15	18.	90	1	15	35.	35	1	5
2.	50	1	5	19.	40	1	34	36.	30	1	3
3.	20	1	32	20.	15	1	15	37.	60	1	5
4.	90	1	15	21.	25	1	3	38.	40	1	3
5.	25	2	18	22.	40	2	7	39.	65	1	2
6.	60	1	21	23.	55	1	10	40.	45	1	5
7.	30	1	13	24.	70	1	12	41.	38	1	7
8.	85	1	19	25.	50	1	7	42.	90	1	5
9.	65	4	34	26.	30	5	2	43.	50	1	4
10.	90	1	28	27.	65	1	5	44.	45	1	5
11.	60	1	28	28.	40	1	3	45.	30	1	5
12.	35	4	31	29.	75	1	2	46.	45	1	8
13.	40	1	17	30.	50	1	5	47.	35	1	11
14.	15	1	31	31.	90	1	10	48.	40	1	12
15.	80	1	31	32.	50	1	7	49.	40	1	5
16.	35	4	22	33.	90	1	5	50.	50	1	4
17.	55	1	22	34.	75	1	7				

*1 – suprabasal; 2 – scattered individual tumour cells; 3 – small groups of tumour cells; 4 – suprabasal and small groups of cells; 5 – suprabasal and individual tumour cells; 6 – scattered individual cells and small groups of tumour cells.

ing or staining of individual cells as well as staining tumor region (basal or suprabasal areas). Quantitative descriptions of staining pattern were made for staining with anti–Ki-67 as percentage of positive cells after counting 500 cells. Additionally, distribution of expression was shown as a diffuse or basal pattern. All results were tested for normality of distribution and percentage staining positive for CK10 and Ki-67 was normally distributed, thus the Kolmogorov-Smirnov test was used for statistical comparison. The p value of <0.05 was considered as significant.

RESULTS

The results showed the range of CK10 expression of total tumor area to be 15%-90% (mean 50.9%) in KA and 2%-30% (mean 13.06%) in SCC. Ki-67 was expressed in a range of 2%-34% (mean 12.4%) in KA and of 12%-98% (mean 39.4%) in SCC (Tables 1 and 2, Fig. 1).

The expression of CK10 was present in an ordered pattern through suprabasal layers in 49 of 50 KAs (98%), whereas the expression Ki-67 showed predominantly staining of basal layers with a varying degree of intensity. In SCC, small groups of tumor cells and scattered individual cells expressed CK10. Proliferating tumor cells in SCC were distributed in a disordered pattern in 30 of 50 SCCs (60%) (Figs. 2-6).

The mean CK10 expression was significantly higher in KA than in SCC ($p<0.001$).

The analysis of Ki-67 expression also showed a much wider range of values and significantly higher median ($p<0.01$) in SCC (Fig. 1).

DISCUSSION

The diagnosis of KA requires distinction from the highly malignant *de novo* SCC, a tumor with a high metastatic potential (1,9). Several features help distinguish KA from SCC but none is absolute. For example, atypical eccrine duct hyperplasia, actinically damaged elastic fibers, increased content of intracytoplasmic glycogen within the epidermis, content of Langerhans cells, and relatively homogeneous staining pattern of involucrins are more

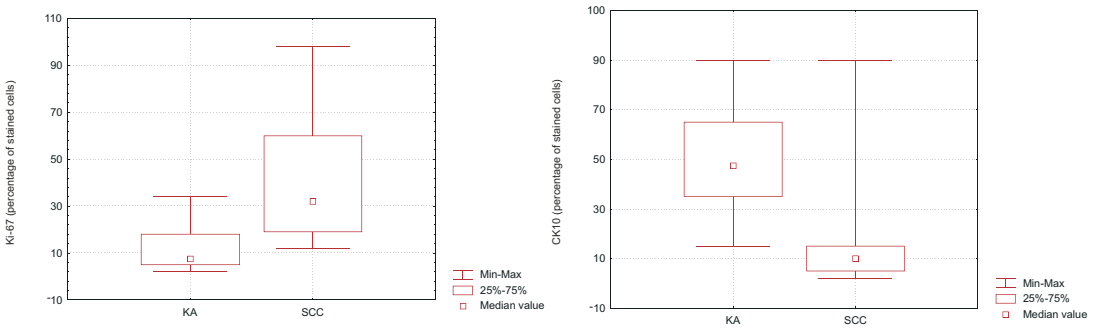

Figure 1. CK10 and Ki-67 expression in keratoacanthomas (KAs) compared with CK10 and Ki-67 expression in squamous cell carcinomas (SCCs) (Box&Whisker Plot).

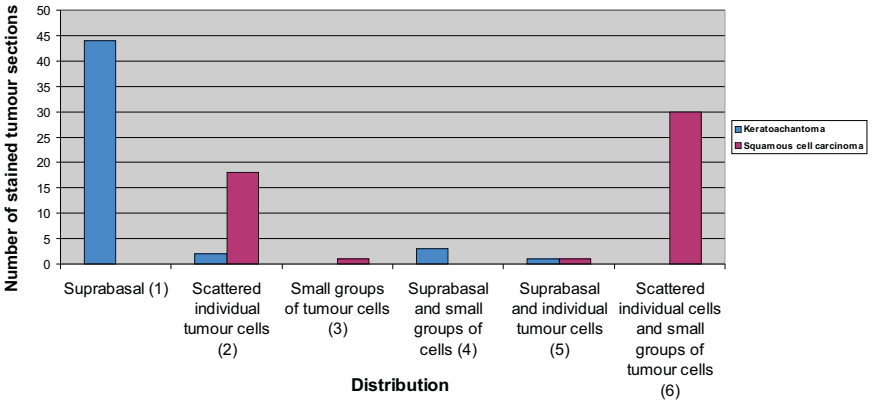

Figure 2. Distribution of CK10 expression in keratoacanthomas (KAs) and squamous cell carcinomas (SCCs).

common in KA (11-15). Additionally, DNA content, quantification of Langerhans cells, nucleolar organizer region enumeration, transforming growth factor alfa, proliferation cell antigen and different oncogene expression were used to distinguish KA from SCC (16-21). The biologic nature of KA has not yet been fully clarified, and there are different opinions on it. Some authors think that KA is a form of resolving SCC, whereas others consider it a distinct entity (22,23). Therefore, attempts were made to distinguish KA from SCC on the basis of expression of different cytokeratins. In normal skin cytokeratin polypeptides are expressed in a different cell type-specific pattern in different layers of the epidermis, and it is known that keratin expression is a phenotypic marker of the stage of keratinocyte differentiation or malignant transformation. Therefore, there is the possibility to diagnose different groups of tumors by their specific cytokeratin patterns (24-31). Our study supports these findings, since our results showed a greater percentage of KA cells than SCC cells to express CK10. CK10 is a high molecular weight keratin that is normally expressed in the suprabasal layer of the epidermis. Assessment of the distribution of CK10 expression in KA and SCC revealed a diffuse suprabasal pattern of expression in KA (similar staining was found in normal skin control slides), whereas in SCC only scattered nests or individual cells showed CK10 expression. We think that this finding could prove useful in clinical practice in making an accurate diagnosis and distinction between KA and well-differentiated SCC. Additionally, Ki-67 count and distribution of stained cells gave us very useful information on the proliferation cell status, which was higher with the diffuse pattern in SCC in comparison with KA, where a low proliferation index and predominantly basal pattern were observed. Similar results were found in different studies of squamous cell carcinoma and other premalignant or benign skin or oral cavity lesions (32-34).

According to our results, KA appears to have a more orderly pattern of differentiation, metabolism and proliferation rate than SCC, which shows a more aggressive behavior and characteristics. Therefore, we think that our results might be helpful in differentiation of these two entities.

Acta Dermatovenerol Croat
2004;12(4):251-256

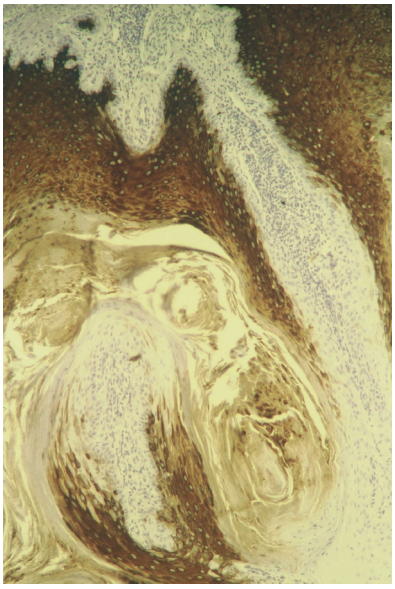

Figure 3. Keratoachantoma. Immunohistochemical staining with anti-CK10 antibody. (Suprabasal distribution), ×200.

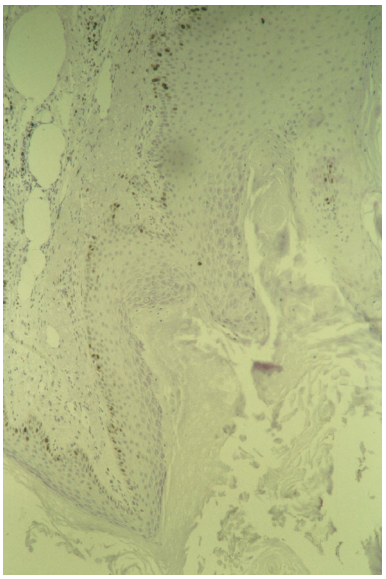

Figure 4. Keratoacanthoma. Immunohistochemical staining with anti-Ki-67 antibody. (Individual cells in basal layer are positive), ×200.

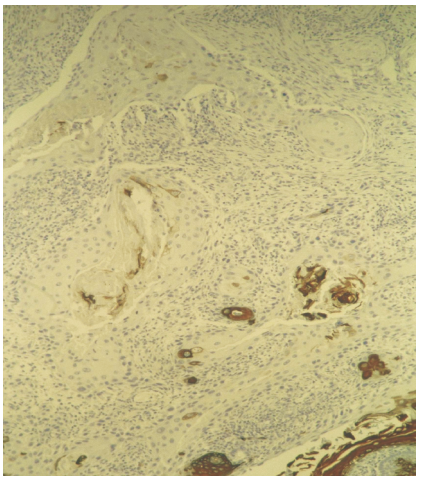

Figure 5. Squamous cell carcinoma. Immunohistochemical staining with anti-CK10 antibody. (Small groups and individual scattered cells are positive), ×400.

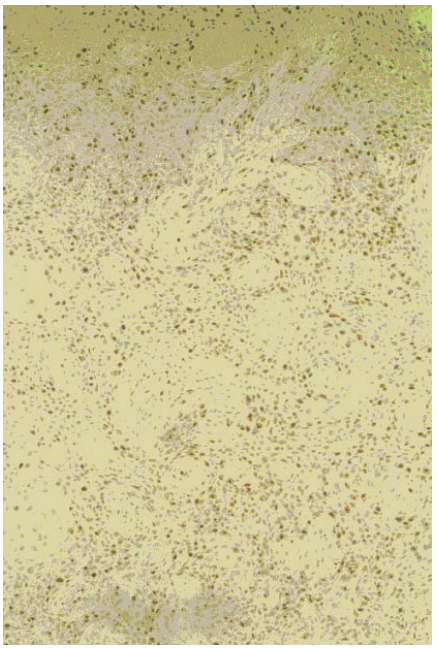

Figure 6. Squamous cell carcinoma. Immunohistochemical staining with anti-Ki-67 antibody. (Majority of tumour cells are in proliferation), ×400.

References

1 Schwartz RA. Keratoacanthoma. J Am Acad Dermatol 1994;30:1-19.

2 Vidović R, Dobrić I. Pseudokanceroze i pseudokancerozne hiperplazije. In: Dobrić I, *et al*. Dermatovenerologija. Zagreb: Grafoplast, 1994;387-91.

3 Lever WF. Pseudocarcinomatosus hyperplasia. In: Lever WF, Shaumburg-Lever G, eds. Histopathology of the skin. 7th ed. Philadelphia: JB Lippincott Company, 1990:559-60.

4 Mackie RM. Tumours of the skin. In: Rook A, Wilkinson DS, Ebling FJG, Champion RH, Burton JL, eds. Textbook of dermatology. Oxford: Blackwell Scientific Publications, 1987:2375-8.

5 Schwartz RA. The keratoacanthoma: a review. J Surg Oncol 1979;12:305-17.

6 Kwa RE, Campana K, Moy RL. Biology of cutaneous squamous cell carcinoma. J Am Acad Dermatol 1992; 26:1-26.

7 Graham JH. Selected precancerous skin and mucocutaneous lesions. In: Neoplasms of the skin and malignant melanoma. Chicago: Year Book, 1976: 69-121.

8 Wick MR, Manivel JC, Millns JI. Histopathologic considerations in the management of the skin cancer. In: Schwartz RA, ed. Skin cancer recognition and management. New York: Springer-Verlag, 1988;246-75.

9 Patel A, Halliday GM, Cooke BE, Barnetson SC. Evidence that regression in keratoacanthoma is immunologically mediated: a comparison with squamous cell carcinoma. Br J Dermatol 1994;131:789-98.

10 Pulch H, Weiss J, Heubner C, Heine M. Differential diagnosis of keratoacanthoma and squamous cell carcinoma: diagnostic value of DNA image cytometry and p53 expression. J Cutan Pathol 1994;21:507-13.

11 Krunic A, Garrod D, Smith N, Orchard G, Cvijetić O. Differential expression of desmosomal glycoproteins in keratoacanthoma and squamous cell carcinoma of the skin. Acta Derm Venerol 1996;76:394 -8.

12 Jordan RCK, Kahn HJ, From L, Jambrosic I. Immunohistochemical demonstration of actinically damaged elastic fibers in keratoacanthomas. J Cutan Pathol 1991;18:81-6.

13 Smoller BR, Kwan TH, Said JW, Banks-Schlegel S. Keratoacanthoma and squamous cell carcinoma of the skin: immunohistochemical localization of involucrin and keratin proteins. J Am Acad Dermatol 1986;14: 226-34.

14 Jensen P, Clausen OP, Bryne. Differences in sialyl-Tn antigen expression between keratoacanthomas and cutaneous sqamous cell carcinomas. J Cutan Pathol 1999;26:183-9.

15 Stephenson TJ, Nichols CE, Otton DWK. Keratoacanthoma contains a higher density of Langerhans cells than well differentiated squamous cell carcinomas. J Pathol 1987;152:238.

16 Kanitakis I, Hoyo E, Hermier C, Chouvet B, Thivolet J. Nucleolar organizer region enumeration in keratoacanthomas and squamous cell carcinomas of the skin. Cancer 1992;69:2937-4.

17 Phillips P, Helm KF. Proliferating cell nuclear antigen distribution in keratoacanthoma and squamous cell carcinoma. J Cutan Pathol 1993;20:424-8.

18 Herzberg AJ, Kerns BJ, Pollack SV, Kinney RB. DNA image cytometry of keratoacanthoma and squamous cell carcinoma. J Invest Dermatol 1991;97:495-500.

19 Batinac T, Zamolo G, Jonjic N, Gruber F, Petrovecki M. P53 protein expression and cell proliferation in non-neoplastic and neoplastic proliferative skin diseases. Tumori 2004;90:120-7.

20 Fisher ER, Mc Coy MM II, Wechsler HL. Analysis of histopathological and electron microscopic determination of keratoacanthoma and squamous cell carcinoma. Cancer 1979;29:1387-97.

21 Klein-Szanto AJ, Barr RJ, Reiners JJ, Mamrack MD. Fillagrin distribution in keratoacanthoma and squamous cell carcinoma. Arch Pathol Lab Med 1984; 108:888-90.

22 Ledo E, Wart MD. Keratoacanthoma and squamous cell carcinoma: a spectrum of the same neoplastic process. Int J Dermatol 1992;31:777-8.

23 Beham A, Requaer S, Soyer HP, Schmid C. Keratoacanthoma: a clinically distinct variant of well differentiated squamous cell carcinoma. Anat Pathol 1998;5: 269-80.

24 Moll R, Moll I, Franke WW. Differences of expression of cytokeratin polypeptides in various epithelial skin tumours. Arch Dermatol 1984;276:349-63.

25 Perkins W, Campbell I, Leigh IM, Mackie RM. Keratin expression in normal skin and epidermal neoplasms demonstrated by a panel of monoclonal antibodies. J Cutan Pathol 1992;19:476-82.

26 Osiecka BJ, Jelen M, Marciniak Z. The evolution of the relationship between malignancy grade and cytokeratin 10 accumulation in human skin squamous cell carcinoma. Przegl Lek 2001;58:435-9.

27 Kurokawa I, Nishijima S, Kusumoto K, Senzaki H, Shikata N, Tsubura A. Trichilemmoma: an immunohistochemical study of cytokeratins. Br J Dermatol 2003:149:99-104.

28 Jensen K, Kohler S, Rouse RV. Cytokeratin staining in Merkel cell carcinoma: an immunohistochmical study of cytokeratins 5/6, 7, 17 and 20. Appl Immunohistochem Mol Morphol 2000;8:310-5.

29 Dusmez Apa D, Aydin O, Polat A, Cinel L, Polat Y, Egilmez R. 34-beta E12 expression in benign and premalignant squamous lesions of skin: relation to cell prolifertion (Ki-67). J Exp Clin Cancer Res 2003;22: 441-5.

30 Ferrar M, Sandison A, Peston D, Gailani M. Immunocytochemical analysis of AE1/AE3m CK14, Ki-67 and p53 expression in benign, premalignant and malignant oral tissue to establish putative markers for progression of oral carcinoma. Br J Biomed Sci 2004;61:117-24.

31 Sigel JE, Skacel M, Bergfeld WF, House NS, Rabkin MS, Goldblum JR. The utility of cytokeratin 5/6 in the recognition of cutaneous spindle cell squamous cell carcinoma. J Cutan Pathol 2001;28:520-4.

32 Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab V, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984;139:1710-5.

33 Tumuluri V, Thomas GA, Fraser IS. The relationship of proliferating cell density at the invasive tumour front with prognostic and risk factors in human oral squamous cell carcinoma. J Oral Pathol Med 2004;33: 204-7.

34 Mekino T, Tatebe S, Goto A, Mihara M, Ito H. Apoptosis and cellular proliferation in human epidermal squamous cell neoplasia. J Cutan Pathol 1998;25: 136-42.