Basic and clinical features of cutaneous squamous cell carcinoma in organ transplant recipients

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CHAPTER 4

p16 Immunostaining in keratinocytic neoplasia in organ transplant recipients: Bowen's disease shows a characteristic pattern

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Abstract

For selecting therapy it is important to distinguish different types of keratinocytic neoplasia. It is sometimes difficult to make histopathologic diagnosis, especially in organ transplant recipients (OTR) who develop numerous lesions.
To investigate p16 immunostaining in different types of keratinocytic neoplasia in OTR, we studied 59 actinic keratoses (AK), 51 Bowen' s disease (BD), 63 squamous cell carcinomas (SCC), 16 benign keratotic lesions (BKL) from 31 OTR patients and 25 controls (eczema and psoriasis). Tissue sections were stained for H&E and p16. We scored intensity, proportion and distribution of p16 positive lesional cells.
In 19% of AK, 92% of BD, 35% of SCC and 12% of BKL more than fifty percent of lesional cells were p16-positive. In 16% of AK, 80% of BD, 18% of SCC and 13% of BKL strong p16 staining was observed. BKL, AK and SCC showed focal and patchy staining, BD showed diffuse pattern with strong staining of all atypical cells. Sparing of the basal layer was predominantly seen in BD. No control specimen showed p16-overexpression.
P16 immunostaining shows a characteristic pattern in BD, but not in AK, SCC and BKL. It appears useful in recognising BD, but not in differentiating between other keratinocytic neoplasia.

Introduction

The spectrum of premalignant and invasive keratinocytic neoplasia includes actinic keratosis (AK), Bowen's disease (BD) and squamous cell carcinoma (SCC). AK can show various grades of intraepidermal dysplasia while BD can be considered as squamous cell carcinoma in situ.[1] For AK and BD a three-tier grading system has been proposed analogous to Cervical Intraepithelial Neoplasia (CIN), named Keratinocytic Intraepidermal Neoplasia, grades KIN I, KIN II and KIN III.[2,3] Both AK and BD can progress into SCC.

Histologic differentiation between pre-cancerous lesions and SCC can be difficult, since the basement membrane can be hard to define. This can be caused by fragmented tissue, small specimen size or severe inflammatory infiltration.[4,5] Correct diagnosis is important since the treatment options depend on the stage of the neoplasia.[6,7] Immunostaining that facilitated differentiation between malignant and premalignant keratinocytic lesions would be of great value.

Immunostaining for the p16 tumor suppressor protein is used as an immunohistochemical marker for anogenital intraepithelial neoplasia induced by high-risk oncogenic human papilloma virus (HPV) genotypes, where its expression is related to the grade of intraepithelial neoplasia.[8-10] P16 up regulation in HPV infected dysplastic cells results from a feedback mechanism consequent on the interaction between the HPV-E7 gene protein and cellular pRB. However, in skin neoplasia the relation between keratinocyte neoplasia and HPV is not as clear.[11] There is evidence that in OTR HPV plays a role in development of keratinocyte neoplasia . However, no significant association between p16 protein expression and the presence of HPV has been found.[12-14] The mechanism of p16 expression in keratinocytic neoplasia is not fully understood. Cutaneous expression of p16 protein has been associated with ultraviolet B irradiation, but this association has not been confirmed.[15-17]

Previous studies using staining for p16 in keratinocytic neoplasia have shown discrepant results and reports on keratinocytic neoplasms in organ transplant recipients are few.[17,18] In the present study we investigated p16 immunostaining patterns in a spectrum of well defined premalignant and invasive keratinocytic neoplasia from organ transplant recipients. The main goal was to find out if differences in p16 immunostaining might be useful to differentiate between these different keratinocytic neoplasia.

Methods

Specimen selection

A total of 189 paraffin-embedded formalin fixed tissue blocks from 31 organ transplant recipients were retrieved from the pathology archive from the Leiden University Medical Center. The blocks were from 59 AK, 51 BD, 63 SCC and 16 benign keratinocytic lesions (BKL). Skin biopsies of psoriasis (n=11) and eczema (n=14) from immunocompetent patients were included as controls.

H&E and p16 immunostaining

All slides were reviewed by a specialized dermatopathologist and classified according to established criteria.[19] Immunostaining was performed using heat-induced epitope retrieval with citrate buffer and a primary mouse monoclonal anti-p16INK4a antibody clone JC8 (Immunologic, Duiven, the Netherlands) followed by visualization using Dako EnVision™ detection system (Dako, Glostrup, Denmark).[20]

P16 scoring

The percentage and distribution of p16 positive lesional cells and the intensity of lesional p16 staining were determined by microscopic evaluation at low scanning power, with visualization of the entire lesion. The proportion of positive lesional cells was scored as: no staining; 1% – 10%; 11% – 50%; and > 50% p16 positive lesional cells. The intensity of lesional cell staining was scored as weak, moderate or strong staining. Positivity was defined as moderate or strong staining for p16 in more than 10 percent of lesional cells.[21]

The study was performed in accordance with the Declaration of Helsinki and the Dutch Code for Proper Secondary Use of Human Tissue, approved by the medical ethics committee of the LUMC.

Results

Actinic keratosis

This group included 27 KIN I and 32 KIN II lesions.

P16 immunostaining showed patchy staining pattern with clusters of p16 positive cells in the suprabasal layers (Figure 1), while the basal layer was frequently negative (Figure 2) .

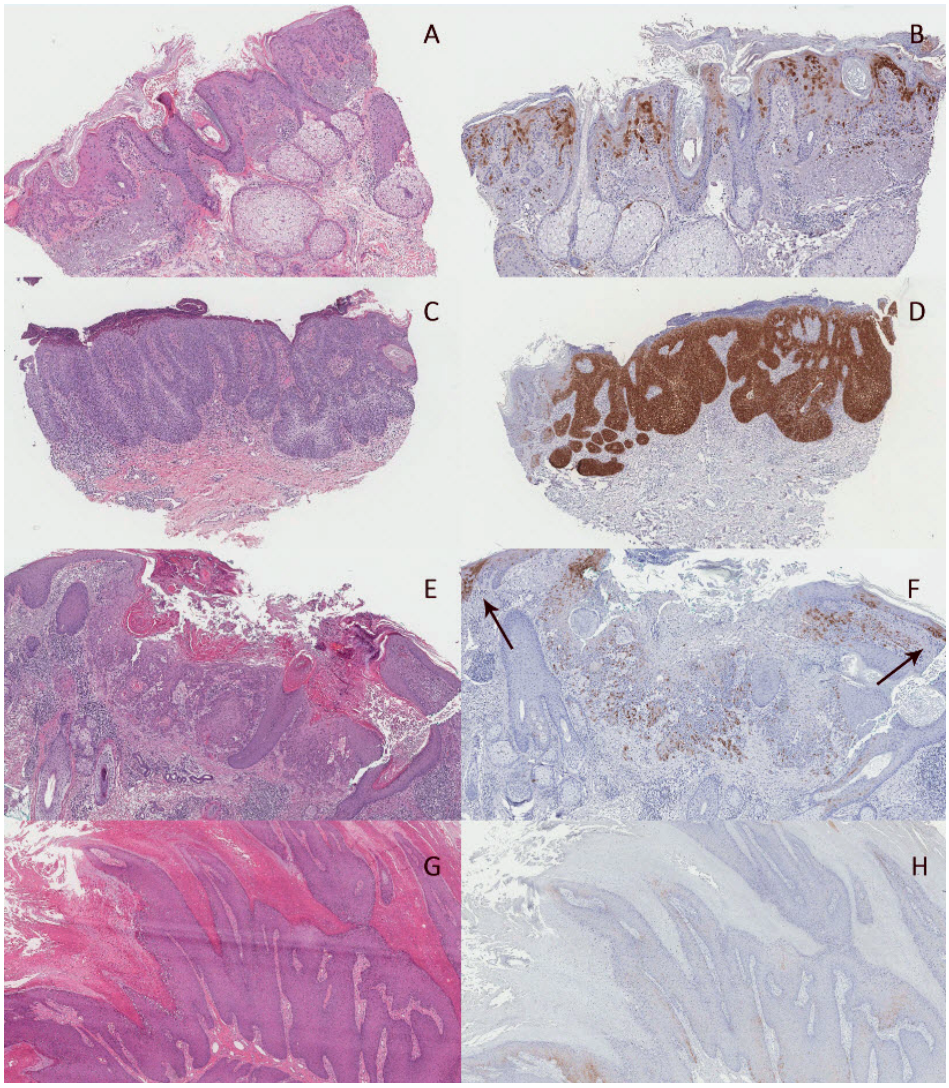

Figure 1. (A-B) Actinic keratosis; keratinocytic intraepidermal neoplasia grade 1 (A) low power view H&E. (B) P16 immunostaining shows a patchy distribution. (C-D) Bowen's disease (C) low power view H&E. (D) P16 immunostaining shows strong staining of lesional cells. (E-F) Squamous cell carcinoma (E) low power view H&E. (F) P16 immunostaining shows a patchy distribution pattern with variable intensity. Note positive cells in the adjacent epidermis compatible with actinic keratosis (ARROW). (G-H) Benign keratotic lesion; case of verruca vulgaris (G) medium power view H&E. (H) P16 immunostaining shows a non-specific pattern with few scattered keratinocytes with low or moderate staining intensity in the lower 1/3 of the epidermis.

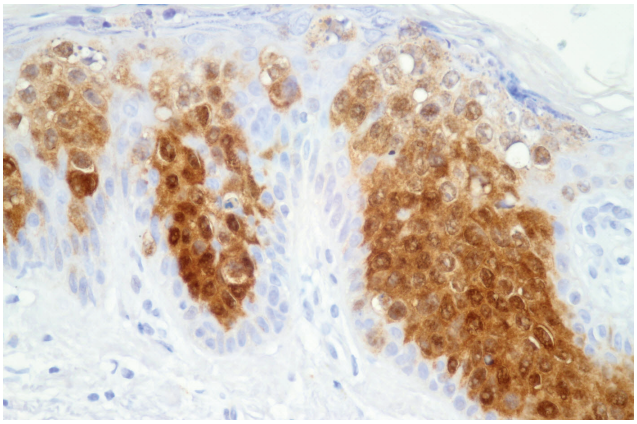

Figure 2.
Actinic keratosis, KINII, showing abundant p16 immunostaining but sparing of the basal layer.

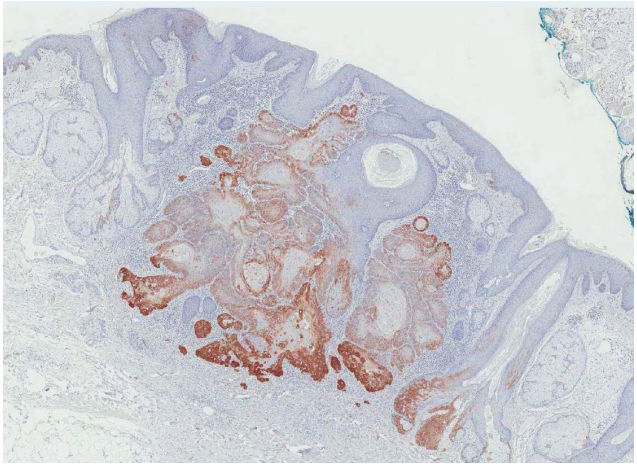

Figure 3.
Well differentiated squamous cell carcinoma with accentuated p16 immunostaining at the invasive peripheral border with the dermis.

Positivity of p16 was seen in 23 of 59 (39%) AK and in 11 of 59 (19%) cases more than 50% of the lesional cells were p16 positive (Table 1). Eight AK were completely negative. The percentage of p16 positive cells were somewhat higher in KIN II than in KIN I lesions, but this difference was not statistically significant. Most AK (63%, n=37) showed moderate intensity of p16 staining in lesional cells.

Bowen's disease.

Diffuse full thickness positivity with strong staining of all atypical cells in the lesional epidermis was seen in 39 of 51 (76%) cases. However, most cases showed focal sparing of the palisaded cells in the basal layer (Figure 1).

Forty-nine of 51 cases (96.1%) showed positivity of p16 immunostaining. In 47 cases (92%) more than 50% of positive lesional cells was seen. None of the BD were completely negative. In 41 of 51 cases (80%) the intensity of p16 staining was strong.

Squamous cell carcinoma

The group of 63 SCC consisted of 45 well differentiated, 14 moderately differentiated, 4 poorly differentiated SCC (Table 1).

Focal staining by p16 in a patchy or scattered pattern, with variable intensity, similar to that observed in AK, was seen in most cases (Figure 1). Also accentuation of the peripheral border of tumor nests was seen in SCC (Figure 1 and Figure 3). Positivity of p16 immunostaining was seen in 40 of 64 cases (62%). Staining of more than 50% of the lesional cells was found in 21 of 64 (33%) cases. Three SCC did not show any positive cells. No (significant) differences were found between well differentiated, moderately and poorly differentiated SCC. In the majority of SCC (75%) the staining intensity was moderate.

Benign keratotic lesion (BKL) and other benign controls

The group of BKL consisted of 5 verrucae vulgaris and 11 seborrheic keratosis.

In most cases a patchy or scattered pattern, with variable intensity was seen (Figure 1). Positivity of p16 was seen in 5 of 16 (31.3%) cases. Only 2 cases, both seborrheic keratosis, showed more than 50 % positive lesional cells. In most cases (75%) the staining intensity was moderate.

In 5 of 14 (36%) eczema and 3 of 11 (27%) psoriasis lesions, a few scattered cells (1-10%) were p16 positive. We observed this in the coil and duct of the eccrine sweat glands, but also some in the seborrheic glands and reactive epidermis. None of these lesions showed positivity of p16. In the other 17 lesions p16 staining was completely negative.

Discussion

This study evaluated p16 immunostaining patterns in different types of keratinocytic neoplasia from organ transplant recipients. P16 staining was most pronounced in BD with positivity (>10% p16 positive lesional cells) in 47 of 51 (92%) cases. The characteristic staining pattern in BD with strong staining of all lesional cells

Table 1. p16 immunostaining in keratinocytic neoplasia.

	Percentage p16 positive cells				Intensity of p16 positive cells*			Positivity**
	0%	**1-10%**	**11-50%**	**>50%**	**Weak**	**Moderate**	**Strong**	
AK (59)	8 (13.6%)	26 (44.1%)	14 (23.7%)	11 (18.6%)	6 (11.8%)	37 (72.5%)	8 (15.7%)	23 (39.0%)
KIN I (27)	4 (14.8%)	14 (51.9%)	8 (29.6%)	1 (3.7%)	3 (13.0%)	19 (82.6%)	1 (4.4%)	8(29.6%)
KIN II (32)	4 (12.5%)	12 (37.5%)	6 (18.8%)	10 (31.3%)	3 (10.7%)	18 (64.3%)	7 (25.0%)	15 (46.9%)
BD (KIN III) (51)	0	1 (2.0%)	3 (5.9%)	47 (92.2%)	2 (3.9%)	8 (15.7%)	41 (80.4%)	49 (96.1%)
SCC (63)	3 (4.8%)	21 (35.0%)	18 (30.0%)	21 (35.0%)	2 (3.3%)	47 (78.4%)	11 (18.3%)	40 (63.5%)
Well differentiated (45)	1 (2.2%)	13 (28.9%)	16 (35.6%)	15 (33.3%)	1 (2.2%)	37 (84.1%)	6 (13.7%)	31 (68.9%)
Moderately differentiated (14)	1 (7.1%)	7 (50.0%)	2 (14.3%)	4 (28.6%)	1 (7.7%)	9 (69.2%)	3 (23.1%)	6 (42.9%)
Poor differentiated (4)	1 (25.0%)	1 (25.0%)	0	2 (50.0%)	0	1 (33.3%)	2 (66.7%)	2 (50.0%)0
BKL (16)	1 (6.3%)	10 (62.5%)	3 (18.7%)	2 (12.5%)	1 (6.7%)	12 (80.0%)	2 (13.3%)	5 (31.3%)
Psoriasis (11)	8 (72.7%)	3 (27.3%)	0	0	1 (33.3%)	2 (66.7%)	0	0
Eczema (n=14)	9 (64.3%)	5 (35.7%)	0	0	0	5 (100.0%)	0	0

*Percentage is calculated in p16 positive cases.
** Positivity of p16 immunostaining was defined as more than 10% moderate or strong staining of lesional cells.
Abbreviations: AK; actinic keratosis, KIN; keratinocyte intraepidermal neoplasia, BD; Bowen's disease, SCC; squamous cell carcinoma, BKL; benign keratotic lesion

accompanied often by sparing of the basal layer, may be considered as a useful adjunct in confirming the diagnosis of BD and in differentiating BD from SCC. In AK and SCC the extent, percentage and intensity of p16 positive cells was much less than in BD, much more variable and did not allow clear-cut distinction between these conditions.

Previous studies of p16 immunostaining in premalignant and malignant keratinocytic lesions showed discrepant results.[12,13,17,18,22-29] Comparison between these different studies is difficult, since different scoring methods were used. Only two studies included lesions from organ transplant patients and their numbers were relatively small.[17,18] Most previous studies of BD and Bowenoid AK report strong p16 staining of the entire epidermis with or without sparing of the basal layer. However, in some studies a considerable proportion of BD, varying between 15% and 40%, did not show positivity of p16. In contrast, in our study staining of less than 10% of the lesional cells was observed in only 1 of 51 (2%) BD cases. Comparison with another study including keratinocytic neoplasms in OTR is impossible, because the results of OTR were not specified.[18] Blokx et al found similar results, with none of the BD (n=14) in OTR negative for p16, while 2 out of 14 BD in immunocompetent patients were p16 negative.[17]

It is reported that progression to SCC occurs in approximately 5 % of BD cases.[19] Such SCC shows poor differentiation and is considered to be of high risk.[30] It is unknown if these SCC show the same extensive p16 immunostaining as observed in BD. The present study contained 1 case of SCC originating from a BD and was poorly differentiated. Remarkably, in this case both SCC and the small remaining area of BD were p16 negative.

Previous studies on p16 immunostaining in AK and SCC reported conflicting results with positivity of p16 varying between 0% and 68 % for AK, and between 10% and 75% for SCC.[12,17,18,22-28] The results of the present study show a gradual increase in the percentage of cases showing positivity of p16 from 34% for KIN I to 50% for KIN II and 63% for SCC. P16 expression by more than 50% of the lesional cells was found in only 1 of 11 (4%) KIN I lesions, compared to 10 of 32 (31%) KIN II lesions and 21 of 64 (33%) SCC. However, staining patterns and intensity were variable in these groups and the differences were not statistically significant, indicating that p16 immunostaining is not useful as a differential diagnostic marker.

For benign keratinocytic lesions (BKL), our results differ from previous reports.[18,23,25,26,28] We grouped the diagnoses of verruca vulgaris and seborrheic

keratosis as BKL in our study. Previous studies that have reported on immunostaining in BKL have studied most frequently seborrheic keratosis and to lesser extent verrucae or benign keratosis not further specified. In two studies, no positivity of p16 in BKL was found.[18,28] Three studies showed positive p16 immunostaining in seborrheic keratosis and benign keratosis in up to 67% of cases.[23,25,26] Those results were consistent with our finding that p16 immunostaining is frequent, but not extensive or strong. All control specimens of psoriasis and eczema did not show positivity of p16 with only focal staining.
Besides extent and intensity of p16 immunostaining, pattern of staining can be informative. This was most distinct in BD. Diffuse full thickness positivity with strong staining of all atypical cells in the lesional epidermis was only seen in BD. Focal staining in a patchy or scattered pattern, with variable intensity and distribution across the lesion was the dominant pattern in AK, SCC and BKL. Two studies have mentioned sparing of palisaded cells in the basal layer while the upper layers show distinct p16 immunostaining as a characteristic finding in BD.[23,29] We observed this pattern predominantly in BD, but it was also seen in AK and SCC. In SCC accentuation of p16 immunostaining at the border of tumor nests with the dermis was observed.

In conclusion p16 immunostaining shows a very distinct staining pattern in most BD, characterised by strong staining of all abnormal cells with sparing of the basal layer cells. This can be useful in confirming a diagnosis of BD. However, the distribution, proportion and intensity of p16 positive cells is very variable in AK, SCC and BKL, suggesting that p16 immunostaining cannot be considered as a useful adjunct in differentiating between these conditions.

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