

ORIGINAL ARTICLES

Epidermodysplasia Verruciformis-Like Cells as Histologic Markers of Immunosuppression: Review of 229 Squamous Cell Carcinomas*

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Abstract. *Introduction.* Epidermodysplasia verruciformis (EV) is associated with greater susceptibility to infection by certain oncogenic subtypes of human papillomavirus (HPV). Among other histologic findings, large, clear, oval or rounded cells (EV cells) are observed in the granular layer in EV, and some authors consider these cells to be markers of immunosuppression.

Material and Methods. We analyzed 229 squamous cell carcinomas (SCC) to determine whether EV cells were present and to assess whether their presence was associated either with localized or cutaneous immunosuppression (tumors with signs of severe chronic actinic damage or severe stasis dermatitis) or with systemic immunosuppression (immunocompromised or elderly patients).

Results. We observed EV cells in 33 SCC. No statistically significant relationship was observed between the presence of EV cells and immunosuppression. We performed polymerase chain reaction in 8 lesions, but the results were not informative as the DNA was denatured.

Conclusions. We found no relationship between the presence of EV cells and localized or systemic immunosuppression, possibly because the sample was inadequate (almost all SCC studied were associated with signs of immunosuppression, irrespective of the presence or absence of EV cells). Further studies will be required to compare lesions associated with immunosuppression with those in which immunosuppression is absent. The presence of EV cells may be the result of cytopathic effects of certain HPV subtypes, such as HPV 5 or 8, but this will need to be demonstrated using techniques such as polymerase chain reaction.

Key words: epidermodysplasia verruciformis, immunosuppression, papillomavirus, solar elastosis.

LAS CÉLULAS TIPO EPIDERMODISPLASIA VERRUCIFORME COMO MARCADOR HISTOLÓGICO DE INMUNODEPRESIÓN: REVISIÓN DE 229 CARCINOMAS ESPINOCELULARES

Resumen *Introducción.* La epidermodisplasia verruciforme (EV) es una enfermedad en la que existe una mayor susceptibilidad genética a la infección por determinados subtipos oncogénicos del virus del papiloma humano (VPH). Entre otros hallazgos histológicos, en la EV suelen observarse unas células claras, grandes, ovaladas o redondeadas (células EV) en la capa granulosa, que según algunos autores se podrían considerar como un marcador histológico de inmunodepresión.

Material y métodos. Hemos estudiado 229 carcinomas espinocelulares (CE) para valorar si presentaban células EV y si se relacionaban con inmunodepresión, tanto localizada o cutánea (tumores con signos de intenso daño solar crónico o con una dermatitis de estasis intensa), como sistémica (pacientes inmunodeprimidos o ancianos).

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Manuscript accepted for publication September 11, 2007.

*2006 August C. Stiefel Award

Resultados. Observamos células EV en 33 carcinomas. No hemos encontrado relación significativa entre la existencia de células EV e inmunodepresión. Realizamos reacción en cadena de la polimerasa (PCR) en 8 lesiones, pero los resultados no fueron valorables porque el ADN estaba desnaturalizado.

Conclusiones. No hemos demostrado relación entre las células EV e inmunodepresión, localizada o sistémica, posiblemente porque la muestra no era adecuada (se podría considerar que prácticamente todos los CE estudiados se asociaban a signos de inmunodepresión, tanto aquellos que presentaban células EV como los que no). Serían necesarios más estudios que comparasen lesiones asociadas a inmunodepresión con otras que no. Es posible que estas células EV sean el resultado de los efectos citopáticos de determinados subtipos de VPH, como el VPH-5 o el VPH-8, aunque sería necesario demostrar esta hipótesis mediante técnicas de PCR o similar.

Palabras clave: epidermodisplasia verruciforme, inmunodepresión, papilomavirus, elastosis solar.

Introduction

Epidermodysplasia Verruciformis

Epidermodysplasia verruciformis (EV) is associated with greater susceptibility to infection by certain oncogenic subtypes of human papillomavirus (HPV), including subtypes 5 and 8 and, less frequently, others including 9, 10, 12, 14, 15, 17, 19 to 25, 36 to 38, 47, and 49. It also tends to be associated with other cutaneous HPV subtypes, including 1, 2, 3, 27, 28, and 77, which cause flat warts and other benign lesions.¹ Clinically, affected patients tend to present large, multiple, flat and common warts that are grouped in plaques and have been present since childhood. In the second or third decade of life, approximately a third of patients begin to present multiple Bowen disease (BD) lesions, actinic keratosis, and multiple squamous cell carcinomas (SCC), mainly in areas exposed to sunlight.^{2,3}

EV-associated HPV subtypes (EV-HPV) are detected in 90% of these tumors (mainly HPV-5 and less frequently subtypes 8, 14, 17, 20, 47). These EV-HPV subtypes are also found in warty lesions, together with cutaneous HPV types, including HPV-3 and HPV-10.¹⁻³ EV appears to be the expression of an abnormality in cellular immunity^{4,5} and constitutes the paradigm of the oncogenic role of HPV, which has a synergistic or potentiating effect on other oncogenic factors such as UV radiation.¹

A wide range of histopathologic findings have been reported for EV.⁶ Findings are occasionally similar to those of flat warts, though they affect almost all the epidermis and have greater cytoplasmic vacuolization. It is also usual to find cytopathic effects caused by HPV (larger and more numerous keratohyaline granules, koilocytosis, papillomatosis, etc).⁷ A characteristic cytopathic effect of EV-HPV is the finding of large, clear oval or round "swollen" cells (EV cells) with a globular appearance and vacuolization or cytoplasmic granules. These swollen cells are usually found in the stratum granulosum and occasionally group

together to form nests.⁶ Dysplastic cells or malignant cell alterations, mainly toward BD or SCC (with similar histology findings to those of BD or SCC, though with a higher degree of dyskeratosis) may also be observed in EV.⁶

Immunosuppression

The number of immunosuppressed patients is increasing: patients with AIDS, transplant patients being treated with immunosuppressants, cancer patients, etc.⁸ Advanced age alone may also be an important immunosuppressant factor.⁹

Furthermore, various studies mention local and systemic immunosuppression that can be caused by chronic exposure to sunlight.¹⁰⁻¹² UV radiation leads to a cascade of biochemical and cellular changes that finally induce immunosuppressant effects.¹⁰⁻¹²

Immunosuppression and Epidermodysplasia Verruciformis

Immunosuppressed patients occasionally develop lesions that are clinically and histologically similar to those associated with EV.¹³⁻¹⁵ Penneys et al¹⁶ reported a relationship between the observation of EV cells in the epidermis (in isolation or grouped in nests) and immunosuppression. The histologic finding of EV cells in the skin can thus be considered as a kind of marker for immunosuppression.

We carried out a study on SCC in which we observed that some tumors were associated with the presence of EV cells and that most of these were either from immunosuppressed patients or from tumors with intense solar elastosis. Based on the study by Penneys et al,¹⁶ we decided to study whether these cases with EV cells were associated with immunosuppression (immunosuppressed patients, elderly patients, or patients with considerable chronic solar damage).

Material and Methods

We studied 229 SCCs excised over a period of 5 years in the Hospital Vega Baja, Orihuela, Alicante, Spain. Although these 229 SCCs came from 199 patients (45 SCCs were excised from 15 patients), in the context of our study, we considered each SCC as a separate case.

SCCs that may have been recurrences of previously excised tumors, those in which there was no observed relationship with the epidermis, those located on the lips, anogenital area, or mucosa, and those for which a suitable analysis of the histologic preparation was not possible (very little tumor-free margin, partial biopsies, curettage and electrocoagulation, etc) were excluded from the study.

Clinical data (age, sex, site, immunosuppression, recurrent SCC, etc) were obtained from the patients' medical histories or from the pathology archive.

For each histologic preparation, we analyzed the region adjacent to the SCC and determined the following:

1. *SCC cells*. Cells similar to those observed in EV: large round or oval balloon-like cells with a granular, grayish cytoplasm, usually located in the stratum granulosum in isolation or grouped in nests.^{6,16}
2. *Other indirect histologic signs of HPV infection*. Larger and more numerous keratohyaline granules (located in the cytoplasm and nucleus), pseudoparakeratosis (rounded nuclei in the stratum corneum), papillomatosis, and/or koilocytosis.⁷ We also determined whether these indirect signs of HPV infection were located in follicular infundibula or in the epidermis between the follicles (similar to a flat wart). Several indirect signs of HPV infection may be found in the same case or signs may be found in both locations.
3. *Solar elastosis*. We assessed the presence of solar elastosis (or basophilic degeneration of collagen) and whether it extended to the superficial, middle, or deep reticular dermis. Measurement of the depth of elastosis may be less objective in specific locations where the dermis is thinner (such as the cheeks and forehead), though the evaluated findings were adapted to each specific case. Because almost all cases of SCC presented solar elastosis, this variable was grouped according to whether elastosis was mild (no elastosis or elastosis involving the superficial reticular dermis), moderate (involving the middle reticular dermis), or intense (involving the deep reticular dermis).
4. *Stasis dermatitis*. We determined whether signs of associated intense stasis dermatitis were another immunosuppression factor.

We considered immunosuppressed cases to be those involving intense solar elastosis (as a result of chronic sun damage), intense stasis dermatitis, and cases from immunosuppressed or elderly patients.

Polymerase Chain Reaction Technique

The polymerase chain reaction (PCR) technique makes it possible to amplify specific sequences of DNA in order to obtain millions of copies of a single fragment of DNA; this provides a sequence of DNA in sufficient quantity to perform a subsequent molecular analysis.¹⁷

We performed PCR on 8 SCCs with clear indirect histologic signs of HPV infection, including 5 cases in which EV cells had been found. If the technique was positive for HPV DNA (mucosal or cutaneous-EV), the specific HPV type was determined (modified single-specific-primer PCR). Two internal controls were used: one to verify that the PCR technique had been performed correctly and another to verify that the DNA in the sample was not denatured (inhibited sample). The results could not be evaluated as the DNA was denatured in all 8 samples; we therefore did not analyze further cases with PCR.

Statistical Analysis

The results were analyzed using the SPSS statistical package, version 12.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

The relationship between qualitative variables and comparison of proportions in independent groups was studied by analyzing contingency tables using the Pearson product-moment correlation and an error-risk of less than 5% was considered significant ($P < .05$).¹⁸

The relationship between continuous variables (eg, age) and qualitative variables was assessed by analysis of variance.¹⁸

A logistic regression analysis was performed to determine whether there was a relationship between the observation of EV cells and other variables (sex, solar elastosis, immunosuppression, preexisting actinic keratosis or BD, indirect signs of HPV infection, etc). Logistic regression analysis makes it possible to calculate the odds ratio (OR) and thus estimate the association of each variable independently, ie, the possibility that a variable confounds or depends on others is ruled out.¹⁹

Doubtful cases or cases that did not allow appropriate analysis of a variable were considered to be nonevaluable and were not included in the statistical analysis of that variable.

Results

We observed EV cells (in isolation or grouped in nests) in 33 lesions (14.73%) (Figures 1-4).

After comparing the SCCs in which EV cells were found with the rest of the SCCs, we found no relationship ($P > .05$) between the presence of EV cells and age, immunosuppression,

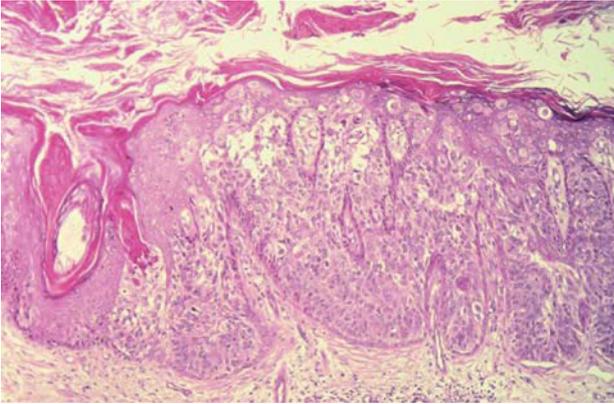


Figure 1. Multiple epidermodysplasia verruciformis cells in a Bowen disease lesion (hematoxylin–eosin, $\times 100$).

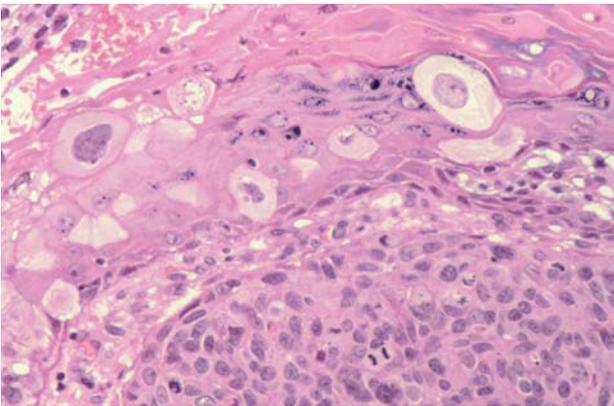


Figure 2. Squamous cell carcinoma in the lower part of the image and epidermodysplasia verruciformis cells in the upper part (hematoxylin–eosin, $\times 400$).

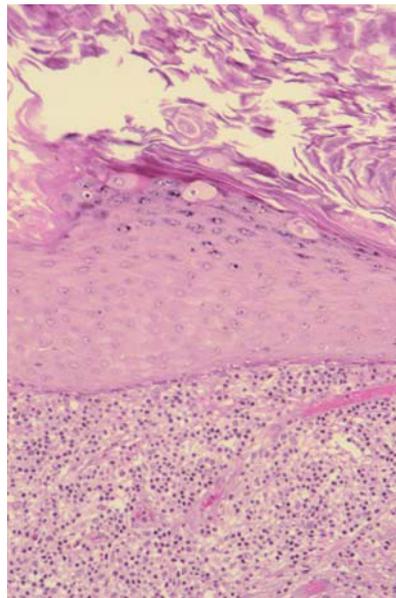


Figure 3. Isolated epidermodysplasia verruciformis cells in the stratum granulosum of the epidermis, with other indirect histologic signs of human papillomavirus infection (hematoxylin–eosin, $\times 200$).

stasis dermatitis, sex, or location in areas exposed to sunlight. Nor did we find a significant relationship between the SCCs where EV cells were present and solar elastosis (Table), even when elastosis was grouped according to whether it was absent or mild, moderate, or intense, or when the sample was divided into groups according to whether immunosuppression was present or not (eg, to determine whether there was a relationship between solar elastosis only in immunocompetent patients because immunosuppressed patients presented a lower degree of solar elastosis).

Other Results

The logistic regression analysis showed an independent relationship between the finding of EV cells and prior BD, with an OR of 10.001 (95% confidence interval [CI], 2.798–35.741), and between EV cells and indirect signs of HPV infection in the epidermis between the follicles, with an OR of 5.717 (95% CI, 1.134–28.817).

Although these 229 SCCs were considered as independent cases in our study, they came from 199 patients: 1 SCC was excised from each of 184 patients and 45 SCCs were excised from the remaining 15 patients (2 each from 9 patients, 3 each from 3 patients, 4 from 1 patient, 6 from 1 patient, and 8 from 1 patient).

The patients with multiple SCCs showed a higher percentage of EV cells: in 9 (20%) of the 45 SCCs we also found EV cells (these 9 SCCs with EV cells corresponded to 6 patients, 2 of whom were immunosuppressed), whereas EV cells were found in 24 (13.04%) of the remaining 184 SCCs. We found no significant differences between these 2 groups ($P > .05$).

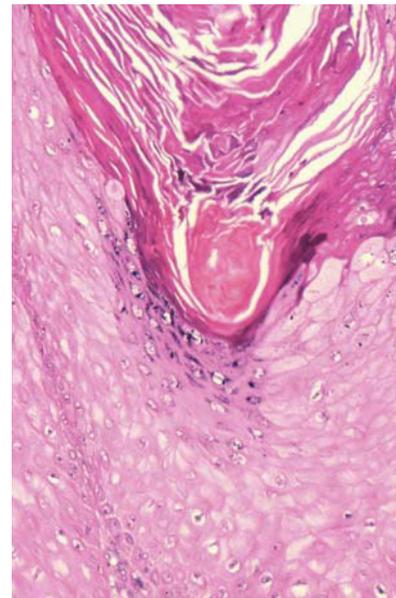


Figure 4. Nests of epidermodysplasia verruciformis cells in an infundibulum, with other indirect signs of human papillomavirus infection (hematoxylin–eosin, $\times 200$).

Table. Epidermodysplasia Verruciformis Cells and Solar Elastosis

		No Solar Elastosis	Surface Reticular Dermis	Middle Reticular Dermis	Deep Reticular Dermis	Total
EV Cells	Yes	1 (3.2)	6 (19.3)	9 (29)	15 (48.4)	31 (100)
	No	5 (2.6)	28 (15)	75 (40.1)	79 (42.2)	187 (100)
Total		6 (100)	34 (100)	84 (100)	94 (100)	218 (100)

^aData are presented as number (%). ^b It was not possible to properly analyze the solar elastosis adjacent to the tumor in 11 lesions and these cases were therefore considered to be nonevaluable.

Abbreviation: EV, epidermodysplasia verruciformis

Discussion

According to the study by Penneys et al,¹⁶ the finding of EV cells is a type of marker for immunosuppression. We found no relationship between EV cells and either local or systemic immunosuppression, though it is possible that this was due to the fact that the sample was not suitable for the reasons that follow.

Sunlight has a marked immunosuppressant effect¹⁰⁻¹² and advanced age may be another immunosuppressant factor.⁹ Patients with considerable chronic sun damage (observable histologically as intense solar elastosis) and those of an advanced age (or both, as older patients also present greater solar elastosis)²⁰ could be considered to be suffering from at least local or cutaneous immunosuppression.²¹ In our sample, most of the patients were of an advanced age and, furthermore, nearly all of the lesions showed intense solar elastosis (extension to the middle or deep reticular dermis), regardless of whether EV cells were present or not. Hence, we can consider that all the patients in our study (including the immunocompetent patients), regardless of whether or not we also found EV cells, were immunosuppressed, and this may explain the lack of differences between the 2 groups. Further studies are required to compare immunocompetent populations (and/or lesions without signs of chronic sun damage) with immunosuppressed patients, patients of advanced age, and patients with intense solar elastosis in order to determine whether the presence of EV cells can be considered as a good marker for local and/or systemic immunosuppression.

We observed EV cells,^{6,16} either in isolation or grouped in nests, in 33 lesions (14.73%). It is possible that these cells are the result of the cytopathic effects of some subtypes of HPV (such as HPV-5 or HPV-8, which are the subtypes most frequently found both in the tumors of patients with EV and in immunosuppressed patients).^{1,21-24} The fact that these EV cells were not found in all the immunosuppressed patients but only in some patients supports the hypothesis that they appear as a result of a specific subtype of HPV, though this requires confirmation with PCR or similar techniques. Our PCR results were inconclusive, probably due to problems with the samples. It has been reported that DNA can degenerate and become undetectable (inhibited

samples) if the lesions remain in formol for more than 24 hours or even if stored for a long time in paraffin^{25,26}; this may have occurred to the SCCs in our study.

The observation of EV cells was linked to BD as a prior lesion and to indirect signs of HPV infection in the epidermis between the follicles (flat warts). Furthermore, in our study these EV cells were observed more frequently in patients from whom multiple SCCs had been excised. All these findings are reminiscent of what occurs in EV patients, who develop multiple BD lesions and flat warts prior to developing multiple invasive SCCs.^{2,3} This also occurs in some immunosuppressed patients.^{8,21,22}

In conclusion, it is possible that the finding of EV cells is a histologic marker for immunosuppression, though further studies are required to confirm this hypothesis. We found no link between the presence of EV cells and immunosuppression, though this may have been due to the fact that the sample was not suitable, as almost all of our cases could be considered to be associated with immunosuppression. Furthermore, it would be of interest to perform studies with PCR, in situ hybridization, or similar techniques²⁷ to determine whether these EV cells appear as a result of the cytopathic effect of some subtypes of HPV, such as HPV-5 or HPV-8.

Conflicts of Interest

The authors declare no conflicts of interest.

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