

ORIGINAL ARTICLES

Clear Cells in Cutaneous Squamous Cell Carcinoma*

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Abstract. *Introduction.* Although few cases of squamous cell carcinoma (SCC) with clear cells have been published, we believe that these cells are often present in SCC.

Material and methods. We studied 249 SCCs, analyzing a number of clinical and histological variables. Various immunohistochemical techniques (immunoperoxidase method) were used to determine whether adnexal differentiation was present.

Results. There were 96 SCCs with a proportion of clear cells of over 25 %. Advanced or established SCCs and SCCs associated with Bowen disease contained a larger proportion of clear cells. We defined 2 histological patterns: a) clear cells around the keratin pearls of SCCs arising from pre-existing actinic keratosis and with indirect signs of human papilloma virus infection in hair follicles; and b) clear cells that simulate adnexal differentiation in lesions arising on pre-existing Bowen disease lesions. There were also 19 carcinomas with true adnexal differentiation.

Discussion. Clear cells are frequently observed in SCC, though large numbers of clear cells are present only in certain SCCs. The appearance of clear cells in SCCs is progressive and they are only present in more advanced SCC. The presence of clear cells is suggestive of adnexal differentiation; however, in the majority of cases, their presence is due to infiltration of normal adnexal structures by the cells of pagetoid Bowen disease. True adnexal differentiation exists only in a small percentage of cases (7.6 % in our study). The histological pattern described as clear cells around keratin pearls practically rules out this differentiation.

Key words: clear cells, squamous cell carcinoma, skin, dermatopathology.

LAS CÉLULAS CLARAS EN EL CARCINOMA ESPINOCELULAR CUTÁNEO

Resumen. *Introducción.* Se han publicado pocos casos de carcinoma espinocelular (CE) de células claras, aunque creemos que es frecuente observar células claras en los CE.

Material y métodos. Hemos estudiado 249 CE. Analizamos distintas variables clínicas e histológicas. Hemos usado diversas técnicas de inmunohistoquímica (método de inmunoperoxidasa) para valorar si existía diferenciación anexial.

Resultados. Observamos 96 CE con más de un 25 % de células claras. Existe más cantidad de células claras en los CE desarrollados o «no incipientes» y en los derivados de enfermedad de Bowen (EB). Describimos dos patrones histológicos: a) células claras alrededor de las perlas córneas del CE, que se asocia a la existencia de queratosis actínica como lesión previa y a signos indirectos de virus del papiloma humano (VPH) en infundíbulos pilosos, y b) células claras que simulan diferenciación anexial, relacionado con EB como lesión previa. También hemos encontrado 19 carcinomas con verdadera diferenciación anexial.

Conclusiones. Es frecuente observar células claras en los CE, aunque solo algunos presentan una gran cantidad. El desarrollo de células claras en los CE sería progresivo y solo aparecería en los CE más desarrollados. Cuando se observan células claras en los CE, se tiende a pensar que existe diferenciación anexial, sin embargo, en la mayoría de casos

se trataría solo de EB de células claras que infiltra estructuras anexiales normales. En un pequeño porcentaje de casos (7,6 % en nuestro estudio) sí existe verdadera diferenciación anexial. Por otra parte, el patrón histológico descrito como células claras alrededor de perlas córneas prácticamente nos descartaría dicha diferenciación.

Palabras clave: células claras, carcinoma espinocelular, piel, dermatopatología.

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Introduction

Kuo¹ described 6 cases of squamous cell carcinoma (SCC) with clear cells as a subtype of SCC that simulates a sebaceous carcinoma. Very few cases of SCC with clear cells (clear cell SCC) have been published (12 or 14 cases²⁻⁸); in our opinion, this subtype of SCC is underdiagnosed and is more frequent than the literature suggests.

Clear cells is a descriptive term that refers to the clear appearance of the cytoplasm in hematoxylin–eosin (H–E) stains. Some authors state that the clear cells are glycogen deposits²; others, based on electron microscopy studies, conclude that these cells are the result of degenerative changes in the cytoplasm (hydropic degeneration,^{1,5} the confluence of vacuoles,⁵ or degeneration of the lysosomes^{9,10}).

Many cutaneous tumors can present clear cells; these tumors may be benign (clear cell acanthoma, trichilemmoma, clear cell hydradenoma, clear cell syringoma, clear cell dermatofibroma, etc) or malignant (clear cell basal cell carcinoma, sebaceous carcinoma, clear cell hidradenocarcinoma, trichilemmal carcinoma, clear cell eccrine porocarcinoma, atypical clear cell fibroxanthoma, clear cell myoepithelial carcinoma, balloon cell melanoma, metastasis of renal carcinoma, etc).²⁻⁵

Some studies suggest that the etiology and pathogenesis of clear cell SCC may be due to adnexal differentiation^{1,8,11}; 3 of the 6 cases reported by Kuo¹ had been previously diagnosed as sebaceous carcinoma. Lee et al¹¹ described a tumor that was indistinguishable from clear cell SCC but that was, in fact, a trichilemmal carcinoma. Similarly, Liegl et al,⁸ after studying 5 of their cases of clear cell SCC located in the penis using electron microscopy, concluded that the tumors were carcinomas with glandular differentiation.

Furthermore, infection by the human papilloma virus (HPV) can cause cytoplasmic vacuolization and changes similar to koilocytes, giving an appearance of clear cells.^{12,13} HPV has also been reported to cause pseudotrilemmal keratinization, giving the false appearance of trichilemmal keratinization in some lesions.^{12,14}

Material and Methods

We analyzed 248 SCCs excised at Hospital Vega Baja, Orihuela, Alicante, Spain, over a period of 5 years.

Although several SCCs had been excised from the same patient, we considered each SCC as an individual case in our study. We defined clear cells as cells that presented a large cytoplasm with an empty or foamy appearance when stained with H–E. We did not consider as clear cells those that presented only weak eosinophilic amphophilic cytoplasmic staining or vitreous keratoacanthoma cells.

We included as clear cell SCC those SCCs containing more than 25% clear cells and specified whether they presented 25% to 50%, 50% to 75%, or more than 75% clear cells.

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.

We studied different clinical and histologic variables for subsequent statistical analysis. The clinical variables studied were the following:

1. Age
2. Sex
3. Location and location exposed to sunlight
4. Patients with known immunosuppression

We obtained the following histologic variables from the analysis of the tumor and adjacent area:

1. Percentage of clear cells: semiquantitative measurement of the number of clear cells observed (cases without clear cells, less than 25%, between 26% and 50%, between 51% and 75%, and more than 75%). This variable was subsequently regrouped into SCCs without clear cells or with less than 25% clear cells, and SCCs with more than 25% clear cells.
2. Whether the clear cells followed a histologic distribution pattern (for this variable, we considered all the SCCs with clear cells, including those with 25% clear cells or less).
3. Possible pre-existing lesion (actinic keratosis, Bowen disease [BD], keratoacanthoma, other).
4. Solar elastosis (basophilic degeneration of collagen) and whether it reached the surface, middle, or deep reticular dermis.
5. Incipient or poorly developed tumor: we considered incipient tumors to be SCCs that had not spread out from the epidermis or the pre-existing lesion and those in which the invading SCC was smaller than the pre-existing lesion.
6. Indirect histologic signs of HPV: presence of large, numerous keratohyalin granules (located in the cytoplasm and nucleus), papillomatosis, pseudoparakeratosis (rounded nuclei in the stratum corneum) and koilocytosis.^{12,15} We also determined whether these indirect signs of HPV were located in the follicular infundibula or in the interfollicular epidermis. Several indirect signs of infection due to HPV can coexist or be found in both locations.

Adnexal Differentiation and Immunohistochemical Analysis

Several immunohistochemical stains with immunoperoxidase were carried out to rule out or confirm adnexal differentiation in some cases. We stained with epithelial membrane antigen (EMA) when we suspected sebaceous differentiation (malignant clear cells with EMA-positive cytoplasm containing microvacuoles,¹⁶ reminiscent of normal sebaceous glands). We stained with carcinoembryonic antigen (CEA) and EMA to determine ductal differentiation (presence of lumen-like formations that stained positive for CEA or EMA, with a cuticle and surrounded by malignant cells or single-cell intracytoplasmic lumens^{16,17}). In these cases, we also stained with cytokeratin 7 (CK-7) to rule out differentiation toward the secretory sudoriferous eccrine or apocrine coil (positive for lumen-like formations¹⁸). Finally, we used CD34 staining when we suspected trichilemmal differentiation (abrupt keratinization and clear cells with a thick cell membrane, positive for CD34 staining¹⁹).

We also used the immunohistochemical stain p16 to study this staining in some SCCs and their pre-existing lesions.²⁰

We used the following as an internal control for these immunohistochemical stains^{16,17}:

1. CEA: excretory duct of normal eccrine or apocrine glands
2. EMA: normal sebaceous glands, the excretory duct of normal glands and some cell membranes
3. CD34: normal vascular endothelia and follicular structures¹⁹
4. p16: eccrine glands and cells of the follicular and sebaceous matrix²⁰
5. CK-7: excretory coils of normal eccrine and apocrine glands¹⁸

Statistical Analysis

The data were processed using the SPSS statistical package, version 12.0 for Windows.

We performed a statistical analysis to determine whether there was a relationship between any of these clinical and pathologic variables and a greater or lesser amount of clear cells, or with a histologic distribution pattern of the clear cells. We first assessed whether there was a relationship between the different variables by means of the χ^2 test and analysis of variance (ANOVA). However, most of the relationships were dependent on other variables, so performed several logistic regression analyses to overcome this situation. These logistic regression analyses made it

possible to calculate the odds ratio (OR) and thus estimate the association of each variable independently, that is, by eliminating the possibility of any variable confounding or depending on others.²¹

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.

Polymerase Chain Reaction Technique

We performed polymerase chain reaction (PCR) on 23 SCCs, 17 of which had indirect histologic signs of HPV infection, 3 had common warts, and 3 had genital condylomata (1 of them with an invasive SCC in the base).

If the technique was positive for HPV DNA (α or mucosal, or β , such as epidermodysplasia verruciformis), 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 (sample inhibition). The results could not be evaluated as the DNA was denatured in 16 of the 23 samples (sample inhibition); we therefore did not analyze further cases with PCR.

Results

We included 248 lesions in our study; of these 248 lesions, 19 were diagnosed as SCC with adnexal differentiation (or adnexal carcinomas). In order to perform a more homogeneous study from a histologic perspective, this subgroup of 19 carcinomas with adnexal differentiation was analyzed separately.

Sample Description

Although the remaining 229 SCCs came from 199 patients (45 SCCs were excised from 15 patients), for the purposes of our study, we considered each SCC as a separate case; 160 SCCs were from men and 69 from women.

The mean age of the patients was 73.4 years and most of the lesions (87%) were located in areas exposed to sunlight; the most frequent location was on the head and neck.

Intense solar elastosis was observed in almost all the SCCs and reached the middle or deep reticular dermis in 182 SCCs (82%). Prior actinic keratosis (AK) was observed in 186 SCCs, BD in 25, and keratoacanthoma (KA) in 39. In some cases, several coexisting prior lesions

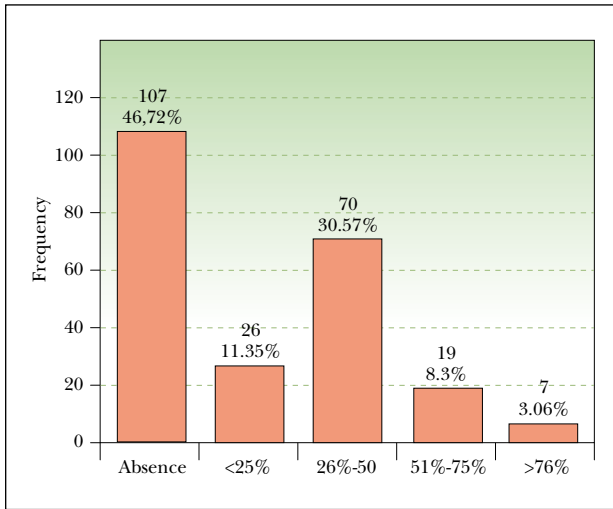


Figure 1. Percentage of clear cells observed.

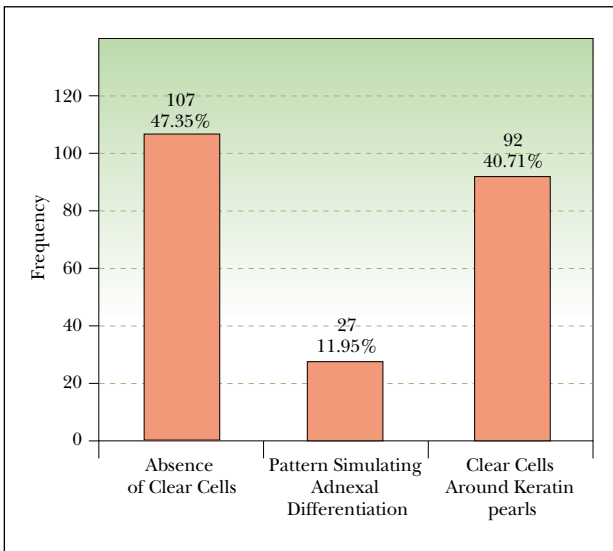


Figure 2. Histologic pattern of distribution of clear cells. SCC indicates squamous cell carcinoma.

were found (AK and KA were observed in 31 cases and AK and BD in 5 cases). We observed indirect histologic signs of HPV infection in 224 of the 229 SCCs. Several of these indirect signs attributable to HPV infection coexisted in most lesions.

Percentage of Clear Cells

We observed clear cells in 122 SCCs (Figure 1). Most of the cases contained 26% to 50% clear cells, whereas only 7 lesions (3.06%) contained more than 75% clear cells. No clear cells were observed in 107 SCCs.

The following independent relationships were observed:

1. SCCs with less than 25% clear cells were associated with prior KA (OR, 15.63; 95% confidence interval [CI], 3.12-76.92) and showed indirect signs of HPV located in the interfollicular epidermis (OR, 6.45; 95% CI, 2.79-14.93).
2. SCCs with more than 26% clear cells were associated with prior BD (OR, 7.550; 95% CI, 2.205-25.854). These SCCs also tend to be developed or nonincipient tumors (OR, 2.56; 95% CI, 1.29-5.10).
3. A relationship with the same variables was observed when analyzing SCCs with or without clear cells (instead of those with more or less than 25% clear cells).

Histologic Pattern

To describe the histologic distribution pattern of the clear cells, we considered all the SCCs with clear cells (including those with 25% clear cells or less). We found 2 histologic patterns (Figure 2): a pattern with clear cells distributed around keratin pearls in the SCC in 92 cases (40.71%) (Figures 3, 4, and 5B) and a pattern with clear cells and apparent adnexal differentiation in 27 cases (11.95%) (Figure 6). Neither of these histologic patterns was found in 3 cases. Finally, no clear cells were observed in 107 cases (47.35%).

We observed the following independent relationships:

1. The histologic pattern described as apparent adnexal differentiation was associated with prior BD (OR, 166.67; 95% CI, 27.04-1000)
2. The histologic pattern described as clear cells around keratin pearls was associated with prior AK (OR, 12.887; 95% CI, 3.375-49.133) and with indirect signs of HPV located in the follicular infundibulum (OR, 9.547; 95% CI, 1.787-50.994).

We found no significant relationship between a histologic pattern or a greater or smaller amount of clear cells and the other clinical and pathologic variables studied; the other significant (χ^2 test) relationships actually depended on the existence of other variables (such as BD or AK), as was shown by the logistic regression analysis.

Histologic Pattern of Apparent Adnexal Differentiation and Immunohistochemical Stain p16

Although the objectives of our study did not include analysis of the findings obtained by staining with the

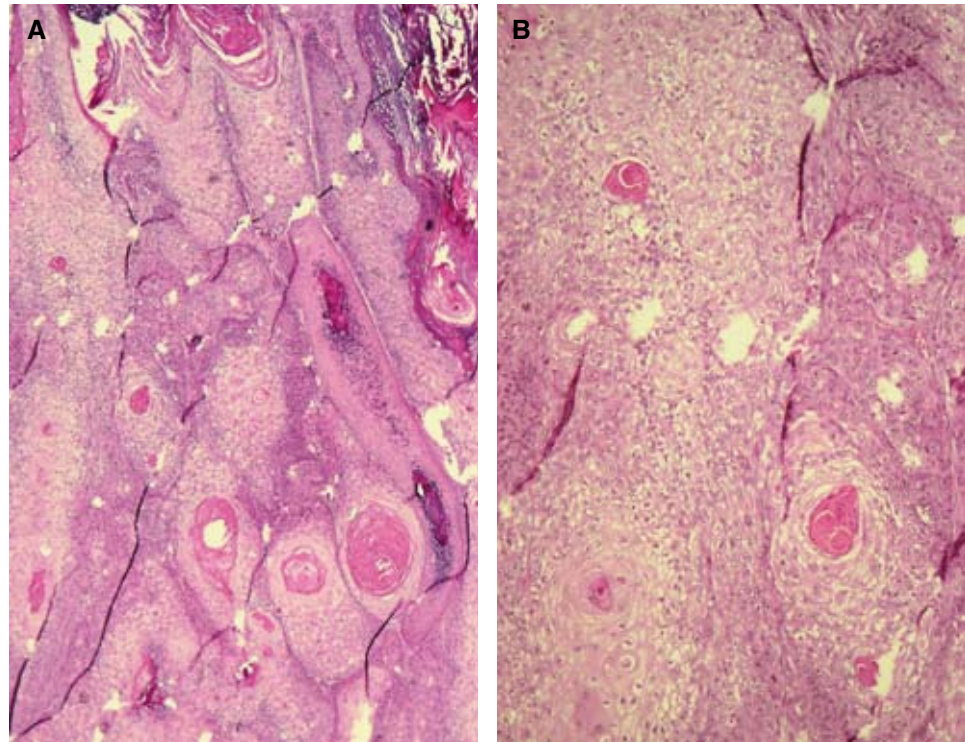


Figure 3. Histologic pattern of clear cells around keratin pearls. A. Hematoxylin–eosin, $\times 40$. B. Hematoxylin–eosin, $\times 100$.

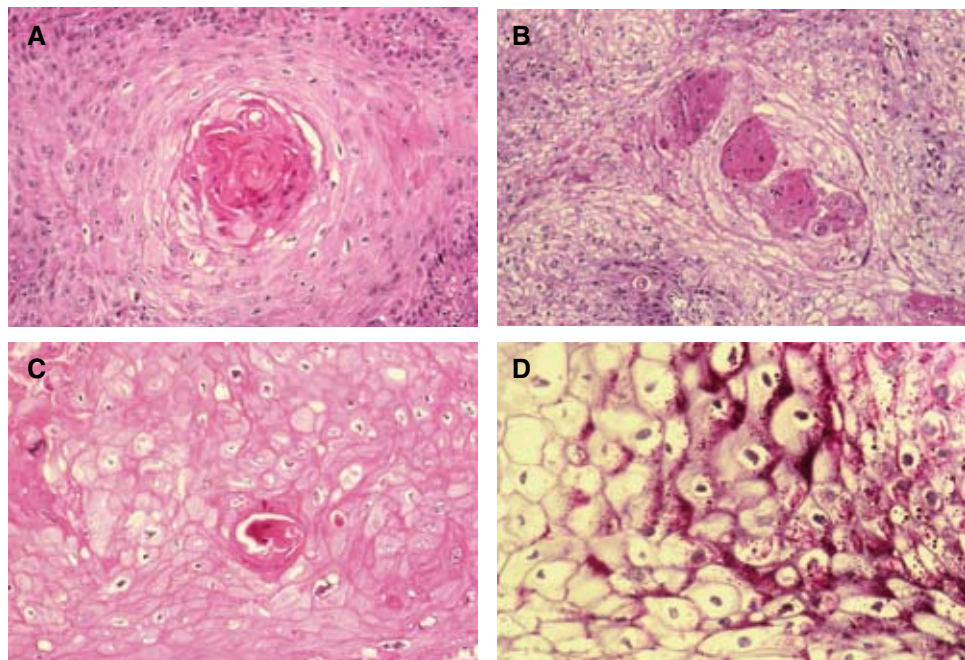


Figure 4. A. Detail of clear cells around keratin pearls (hematoxylin–eosin, $\times 200$). B. Squamous cell carcinoma (SCC) in the axilla, similar to the histologic pattern of clear cells around keratin pearls (hematoxylin–eosin, $\times 200$). C. SCC on the glans, also similar to this histologic pattern. Koilocytosis (hematoxylin–eosin, $\times 200$). D. Koilocytosis in a clear cell SCC. Positive PAS stain showing the perinuclear halo (PAS, $\times 400$).

immunohistochemical stain p16, in either the SCCs and their principal pre-existing lesions (AK, KA, and BD); we can however report that a strong positive reaction to staining with p16 was observed in 25 of the 28 lesions studied: 14 of 16 were positive for BD and 11 of 12 were positive for Bowenoid AK. The invasive SCCs derived

from these lesions that stained positive for Bowenoid AK also stained positive with p16.

Both the BD cells and the AK cells invaded normal adnexa; this invasion was shown by staining with p16 (Figure 5A). Of the 27 SCCs with a histologic pattern of apparent adnexal differentiation, 22 were normal adnexa

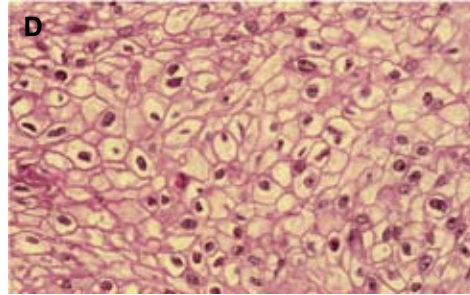
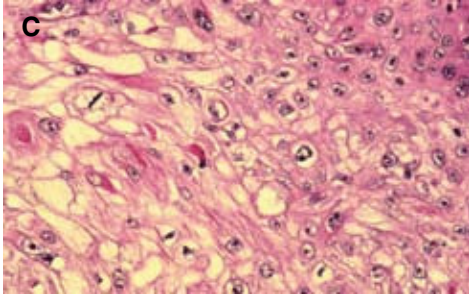
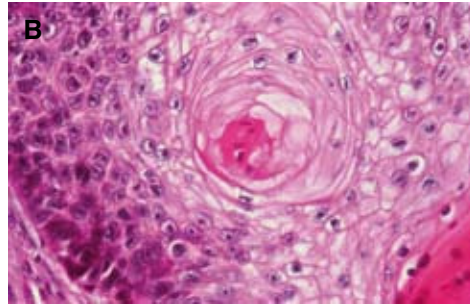
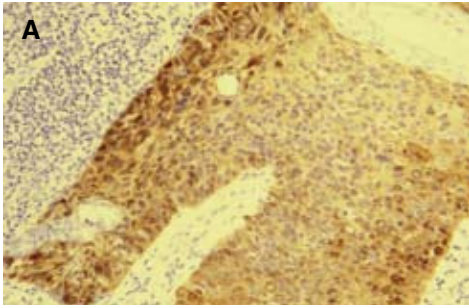


Figura 5. A. Positive staining with p16 in Bowen disease infiltrating normal adnexal structures (p16, $\times 200$). B. Pattern of clear cells around keratin pearls, similar to trichilemmal differentiation (hematoxylin–eosin, $\times 400$). C. Clear cells in SCCs, simulating sebaceous cells (hematoxylin–eosin, $\times 400$). D. Clear cells with a thick cell membrane, similar to trichilemmal cells (hematoxylin–eosin, $\times 400$).

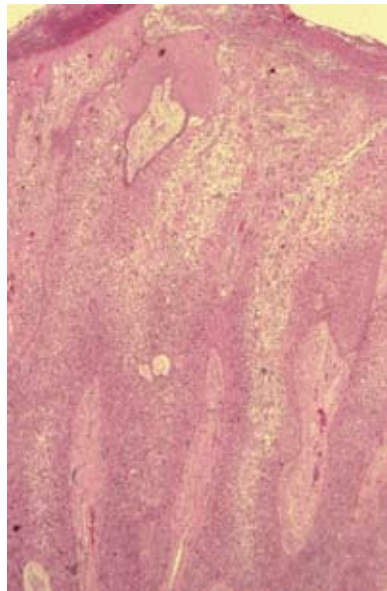


Figura 6. Pattern of clear cells with apparent adnexal differentiation derived from Bowen disease (hematoxylin–eosin, $\times 40$).

invaded by the Bowenoid cells (this was observed following staining with p16, which stains positive for Bowenoid cells). The other 5 SCCs that presented this histologic pattern were tumors with doubtful or apparent adnexal differentiation that could not be confirmed after staining with different immunohistochemical stains (CEA, EMA, CD34, CD10, and CK-7). These lesions were therefore not included in the subgroup of carcinomas with adnexal differentiation.

Carcinomas With Adnexal Differentiation

In the subgroup of 19 carcinomas that did show adnexal differentiation, this differentiation (luminal-ductal, sebaceous, trichilemmal) was suspected following staining with H–E and was confirmed using immunohistochemical staining. We found 12 tumors with luminal and sebaceous differentiation; we therefore concluded that it was sebaceous-apocrine differentiation, based on the common embryologic origin of the folliculosebaceous-apocrine unit.²² Another 5 carcinomas with luminal differentiation were diagnosed as eccrine porocarcinoma (2 cases) and carcinomas with differentiation toward the sweat secretory coil (3 cases) (positive for CK-7).¹⁸ We also found a trichilemmal carcinoma and a malignant proliferating trichilemmal cyst, both of which stained positive for CD34.¹⁹

Most of these adnexal carcinomas had a large amount of clear cells (more than 26% in 15 carcinomas and more than 51% in 10); these figures are significantly higher when compared with the other SCCs in our study ($P=.002$ and $P=.000$). Fifteen of these cases corresponded to the histologic pattern of adnexal differentiation, 1 to the pattern of clear cells around keratin pearls, and 1 case had no clear cells; 2 cases showed no well-defined histologic pattern.

In terms of the other clinical and pathologic variables, we compared this subgroup of 19 carcinomas with the other SCCs (229 lesions) but observed no significant differences. The only difference was that none of these carcinomas was incipient ($P<.001$).

Periodic acid-Schiff and Periodic acid-Schiff Diastase Stain

We studied only 17 clear cell SCC that had already been stained with periodic acid-Schiff (PAS) and PAS diastase stains; 13 cases (76.5%) were positive for PAS staining (negative for PAS diastase staining). We observed diffuse positive cytoplasmic staining in most of the tumors in 9 of the 11 Bowenoid lesions (6 BD and 5 Bowenoid AK). The other tumors were 4 of 6 clear cell SCC with a histologic pattern of clear cells around keratin pearls, though positive staining in these cases was focal (only in some areas of the tumor) and in the peripheral area of the cell cytoplasm, highlighting the perinuclear halo of these cells (koilocytosis)^{12,15} (Figure 4D).

Discussion

Sample Description

The clinical data of patients with the SCCs studied are similar to those of other studies: predominantly elderly male patients (the mean age of 73.4 years is slightly higher than that of other studies²³). Location in areas exposed to the sun (87% in our sample and approximately 90% in other studies^{23,24}) and the fact that most lesions were located on the head and neck are also similar findings to those of other studies.²⁵ Similarly, in our study, the predominant pre-existing lesion is AK (we observed AK in isolation or with other prior lesions in 82.67% of cases), while other studies found AK in the epidermis adjacent to the invading SCC in 72%²⁶ or 82.2%²⁷ of cases. The presence of AK as the predominant lesion and the considerable solar elastosis observed may indicate the type of exposure to sunlight in these patients: according to other studies, these SCCs are associated with chronic, sustained exposure or occupational exposure to the sun.²⁸

We found histologic signs attributable to HPV infection in almost all the SCCs; this high incidence of signs of HPV infection may be related to the observed signs of intense chronic sun damage and the resulting (at least localized) immunosuppression that can be associated with this chronic sun damage.^{29,30} Furthermore, many of these indirect signs of HPV infection could correspond to cutaneous, nononcogenic HPV types. These findings may also support the possible role of HPV in the oncogenesis of SCC, particularly in immunosuppressed patients (and in immunocompetent patients with intense chronic sun damage, as in the cases reported in our study).^{29,30} Unfortunately, we were not able to confirm HPV infection using PCR, possibly due to deficiencies in the technique used or in the samples (most of the samples had denatured DNA).³⁰

Percentage of Clear Cells

There are very few cases of clear cell SCCs in the literature; Kuo¹ studied 229 SCCs and found only 6 clear cell SCCs (2.6%). These 6 cases were almost completely composed of clear cells because cases with focal clear cells were not considered as clear cell SCCs. In our study (not including the 19 carcinomas with adnexal differentiation), we found clear cells in 122 SCCs (53.3%). Nevertheless, we only found 26 SCCs (11.36%) with more than 50% clear cells and only 7 (3.06%) with more than 75% clear cells. This proportion of tumors with a large amount of clear cells is similar to that found by Kuo.¹ We believe that clear cells are common in SCCs, though only some SCCs present a large number of clear cells.

The invasive SCCs derived from BD present a larger proportion of clear cells. BD with clear cells is caused by accumulations of glycogen,³¹ as in some of our cases (shown by PAS staining). SCCs that appear at the base of some KAs or those with signs of HPV infection in the interfollicular epidermis present fewer clear cells (in fact, they tend to be SCCs with no clear cells). During observation of the histologic samples, we found that the SCCs derived from KA and those with indirect signs of HPV infection similar to a flat wart (signs of HPV infection in the interfollicular epidermis) or a common wart tended to be classic or keratinizing SCCs with few or no clear cells.

The presence of few or no clear cells in incipient SCCs and a greater proportion of clear cells in advanced SCCs suggests that the development of clear cells in the SCCs (whether due to the accumulation of glycogen, cytopathic effects of HPV, or other causes) is a progressive process that requires time and only occurs in more developed SCCs.

Histologic Pattern of Clear Cells Around Keratin Pearls

In our study, most of the SCCs with clear cells correspond to the histologic pattern described as clear cells around the keratinizing areas and/or keratin pearls (92 lesions [40.71%]) (Figures 3, 4, and 5A). This histologic pattern is associated with the presence of histologic signs of HPV infection in the follicular infundibula and AK as a prior lesion. These associations may indicate that there is a relationship between this histologic pattern and solar radiation, as an increase in HPV in the hair follicles (and therefore in the infundibula) in association with exposure to sunlight has been reported,^{32,33} and AK is usually located in areas exposed to the sun.³¹ However, 2 of the cases with this histologic pattern were located in areas not exposed to the sun (axilla and torso) and therefore did not show solar

elastosis but did have clear signs of HPV infection (Figure 4B). Furthermore, this pattern is histologically similar to basaloid SCCs of the vulva, which have been shown to be associated with HPV.¹³ To verify this, we analyzed 9 SCCs located in the anogenital region and 7 of them were similar to those with a histologic pattern of clear cells around keratin pearls (Figure 4C). All of this suggests that the presence of HPV may be an essential factor for the development of this histologic pattern, whereas AK or chronic solar damage would be additional factors.

This histologic pattern of clear cells around keratin pearls may bear some similarity to trichilemmal keratinization or to changes observed in trichilemmoma^{16,17,19} (Figure 5B and 5D). The cases of doubtful trichilemmal differentiation can be confirmed or ruled out by means of CD34 staining.¹⁹ It has been reported that HPV can cause pseudotrilemmal keratinization, giving a false trichilemmal appearance to some lesions.^{12,14} It is possible that the trichilemmal appearance of some SCCs that present this histologic pattern may be caused by the presence of HPV. In our study, this pattern is associated with the presence of indirect signs of HPV infection in the infundibula, thus supporting this hypothesis. Furthermore, PAS staining was positive in 4 of the 6 clear cell SCCs that presented this histologic pattern (positive staining around the perinuclear halo, in areas of koilocytosis in the tumor), which supports the presence of HPV infection in these clear cell SCCs (Figure 4D). Most of the clear cells in our study are associated with this histologic pattern and are the result of the cytopathic effects of certain types of HPV (koilocytosis or cytoplasmic vacuolization,^{12,15} or pseudotrilemmal keratinization^{12,14}).

Histologic Pattern of Apparent Adnexal Differentiation Adnexal Carcinomas

We reported this pattern in 27 clear cell SCCs that appeared to have adnexal differentiation (not subsequently confirmed), based on the finding of lumens surrounded by malignant cells or areas with apparent sebaceous or trichilemmal differentiation^{16,17} (Figure 6). After immunohistochemical staining with p16 (positive in most of the Bowenoid lesions, as in other studies³⁴), we found that practically all of these cases corresponded to BD or Bowenoid AK infiltrating normal adnexal structure and, therefore, were only simulating adnexal differentiation (Figure 5A). Although this observation might seem logical, we found that, in some tumors stained with p16, the Bowenoid cells invaded sweat ducts surrounding the normal lumen (this type of invasion had initially been interpreted as possible luminal differentiation). Staining with p16 made it possible to differentiate between true

adnexal differentiation and Bowenoid cells infiltrating normal adnexa.

Moreover, the clear cells appear to be sebaceous or trichilemmal cells in some cases (Figure 5C and 5D); this histologic pattern is associated with BD, which has a greater proportion of clear cells. All this also favors the histologic pattern of apparent adnexal differentiation.

The SCCs that appeared to have adnexal differentiation were almost all BD or Bowenoid AK, as these lesions are associated with a larger proportion of clear cells and infiltrate normal adnexal structures. However, the 19 carcinomas which did show adnexal differentiation also presented a larger number of clear cells. Thus, when a reasonably large number of clear cells are observed in an SCC, this may be an example of BD simulating adnexal differentiation, but true adnexal carcinoma must be ruled out.

In our study, observation of the histologic pattern of clear cells around keratin pearls practically rules out adnexal differentiation (independently of the amount of clear cells observed): of the 92 SCCs that presented this histologic pattern, adnexal differentiation was suspected in only 3 cases (3.26%) but not subsequently confirmed; in the subgroup of 19 adnexal carcinomas, only 1 of them presented findings similar to the pattern of clear cells around keratin pearls. Where histologic signs of probable luminal, trichilemmal, and/or sebaceous differentiation are present, immunohistochemical staining should be performed to confirm or rule out the finding.¹⁶⁻¹⁹ The clinical importance of this is that carcinomas with adnexal differentiation may show aggressive clinical behavior, with a tendency toward recurrence or metastasis (such as porocarcinoma³⁵ or sebaceous carcinoma^{36,37}) or they may be associated with an excellent prognosis despite the histologic appearance (such as trichilemmal carcinoma).³⁸

In our study, 19 (7.66%) of the 248 lesions analyzed were carcinomas with adnexal differentiation. From a methodologic point of view, the decision to study these 19 carcinomas as an independent subgroup may be questionable, though we did not want to analyze these adnexal carcinomas with the other SCCs. Because they are histologically distinct tumors, we believed that the relationship with the various study variables could also have been different and have influenced the results of our study. We subsequently showed that there were no significant differences between this subgroup and the other SCCs with respect to most of the clinical and pathologic variables, except for the fact that these tumors had a higher proportion of clear cells. This could be because none of these tumors was incipient (as with the clear cell SCCs, the development of clear cells in these tumors also requires time and, therefore, the most developed or least incipient tumors would have a larger number of clear cells).

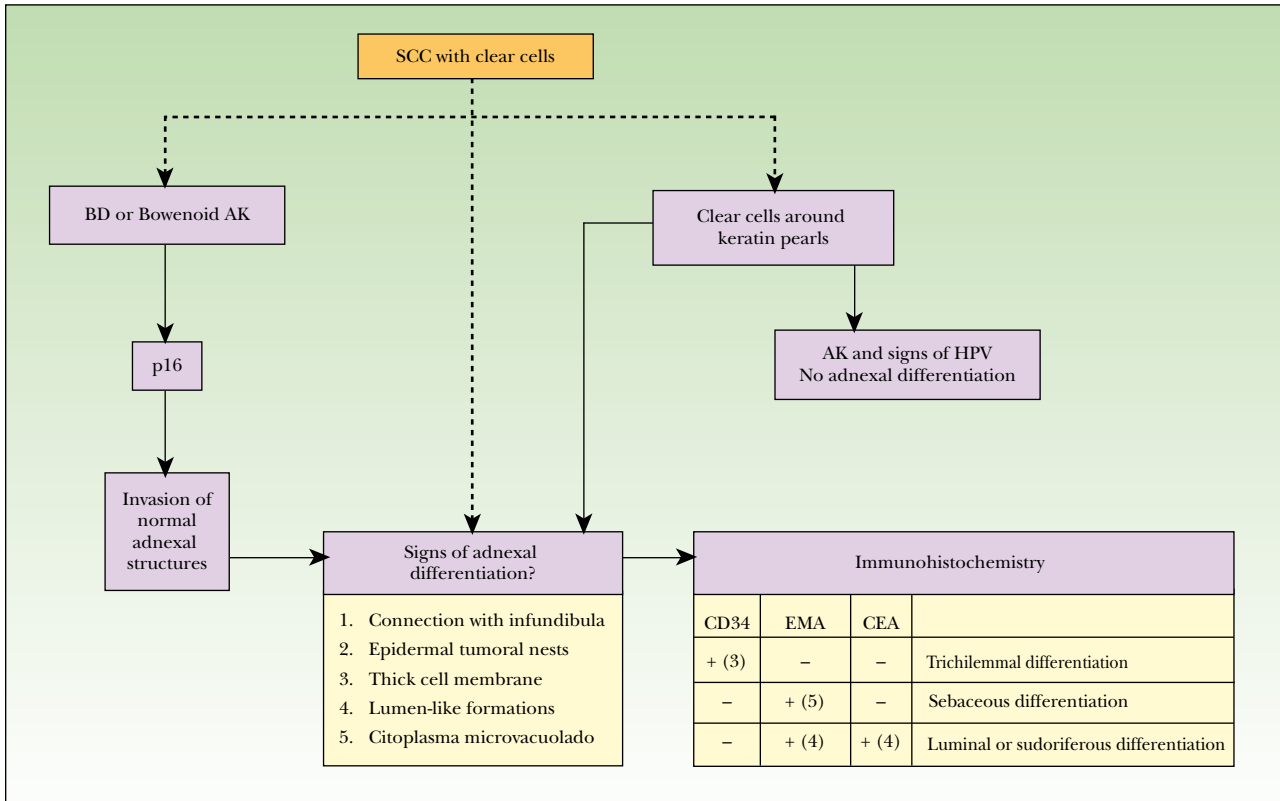


Figure 7. Diagnostic algorithm in squamous cell carcinomas (SCC) with clear cells. AK indicates actinic keratosis; BD, Bowen disease; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; HPV, human papilloma virus.

In summary, due to their histologic appearance, finding clear cells suggests adnexal differentiation in an SCC. Most cases, however, do not involve adnexal differentiation, but are cases of BD infiltrating normal adnexa, as we observed after immunohistochemical staining with p16; furthermore, BD is associated with a larger number of clear cells, thereby enhancing the adnexal appearance. Moreover, the histologic pattern described as clear cells around keratin pearls practically rules out adnexal differentiation, independently of whether a larger or smaller number of clear cells is observed. We found 19 carcinomas (7.66%) with true adnexal differentiation and with a larger amount of clear cells than the other SCCs. Because the clinical prognosis of these carcinomas with adnexal differentiation may be different than that of SCCs,³⁵⁻³⁸ we believe that it is important to rule them out.

Finally, further studies with a larger sample size, and preferably of multicenter and prospective design, are needed to verify our results.

Figure 7 shows a possible diagnostic algorithm for SCCs with clear cells.

Conflicts of Interest

The authors declare no conflicts of interest.

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