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# Cytokeratin Profile of Basal Cell Carcinomas According to the Degree of Sun Exposure and to the Anatomical Localization

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**Abstract:** Basal cell carcinoma (BCC) seems to originate from ultraviolet light-induced mutations involving the bulge or the outer sheath of the hair follicle cells. However, the etiopathogenic mechanisms involved in the development of these tumors in nonphotoexposed and in hairless areas remain unclear. The cytokeratin (CK) profile (including CK5/6, CK7, CK14, CK15, CK17, and CK19) from a series of different BCC subtypes developing in sun-exposed and non–sun-exposed areas, including hairless regions, was evaluated. The authors have observed that CK7 expression in BCC is associated with the anatomical localization of the tumor and its sun-exposition, but not with other factors such as histological subtype. The expression of this CK is higher in BCCs located in non–sun-exposed and nonhairy areas, such as the vulvar semimucosa and the nipple. Because CK7 is a marker of simple glandular epithelia, the authors suggest a glandular origin for BCCs located in hairless and nonphotoexposed areas.

**Key Words:** basal cell carcinoma, cytokeratins, hair follicle, sebaceous gland, photoexposition

(*Am J Dermatopathol* 2017;0:1–7)

## INTRODUCTION

Basal cell carcinoma (BCC) is the most common malignancy in whites. BCC often arises on sun-exposed areas such as the face and upper trunk and shows slow growth and a variable locally aggressive behavior.[1] The cellular origin of the BCC is not completely elucidated, but development of BCC from both the *stem cell* of the outer sheath of the hair follicle or hair bulge has been hypothesized.[2] Some authors have suggested that a subset of BCC may arise from the interfollicular epithelium.[3,4] In patients with Gorlin syndrome (nevoid BCC syndrome), germ-line mutations in the *PTCH1* gene have been demonstrated and molecular somatic mutations in the *PTCH1* gene and other genes encoding proteins of the hedgehog pathway have also been detected in sporadic BCC.[5–8] However, somatic mutations in BCC usually show the characteristic pattern of UV-induced mutations.[9]

Cytokeratins (CK) constitute intermediate filaments of epithelial cells, forming part of the cellular cytoskeleton.[10] Malignant tumor cells tend to retain the CK expression pattern of the original cells and can be used as potential markers to determine either the origin of a tumor and to confirm the nature of metastatic cancer of unknown origin.[11] Several studies have been performed to evaluate CK expression in BCC.[12–16] However, in large series of tumors, no clear-cut differences regarding the expression pattern in different clinical and histopathological variants of BCC have been detected. However, as far as we are concerned, no previous studies have evaluated the CK expression profile in BCC in relation to both the exposure to sunlight and anatomic location.

## MATERIALS AND METHODS

### Biopsies

Sixty-seven skin biopsy samples were retrospectively obtained from the Dermatology Pathology files at the Arnau de Vilanova University Hospital (Hospital Universitari Arnau de Vilanova), and Biomedical Research Institute's Biobank (IRBLleida Biobank) in Lleida, Spain. Both institutions were authorized by the Department of Health of the Government of Catalonia as from April 29, 2013, and registered on the National Register of Biobanks of the Carlos III Health Institute (Spain) under reference number B.0000682. The Biobank guarantees the traceability and quality of the samples and the consent process undertaken in accordance with the protocols approved by the Local Ethical Committee following the basic principles (respect for the individual), operational risk-management (risk–benefit), and guidelines (good clinical

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The authors declare no conflicts of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.amjdermatopathology.com).

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practice) of the Declaration of Helsinki (World Medical Association, 1964). The included samples had been selected according to the area involved, including only samples of BCC developing in chronic or intermittent sun-exposed areas and in non–sun-exposed areas.

The following data were recorded for each patient with BCC: sex, age, solar elastosis, tumor differentiation, and tumoral localization (see **Tables 1–3**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A59). Thirty BCCs had developed in a chronic photoexposed area (face) (group 1); 22 BCCs arising in an intermittent photoexposed area (upper trunk) (group 2), including a case of BCC on a man's nipple, and 15 BCCs developing in nonphotoexposed locations (group 3), including 5 vulvar BCCs (hairy area), 4 BCCs located on the inner part of the labia majora (semimucous membrane) (a hairless area), 1 on the perineum, 1 on the female mons pubis, 1 on the female breast, and 3 on axillae.

## Histopathological Evaluation

Following a systematized protocol, a panel of histopathological features was blindly evaluated by 2 independent observers. BCCs were classified as superficial, nodular, or infiltrating BCC. Tissue blocks were sectioned at a thickness of 3 μm, dried for 1 hour at 65° before pretreatment procedure of deparaffinization, rehydration, and epitope retrieval in the Pre-Treatment Module, PT-LINK (DAKO) at 95°C for 20 minutes in ×50 Tris/EDTA buffer, pH 9. Before staining the sections, endogenous peroxidase activity was blocked. Anti-CK monoclonal antibodies against CK5/6 (DAKO, Glostrup, Denmark), CK7 (DAKO, Glostrup, Denmark), CK14 (Leica, Newcastle, United Kingdom), CK15 (Leica, Newcastle, United Kingdom), CK17 (DAKO, Glostrup, Denmark), and CK19 (DAKO, Glostrup, Denmark) were evaluated (Table 1).[17] After incubation, the reaction was visualized with the EnVision FLEX Detection Kit (DAKO, *Glostrup, Denmark*) using diaminobenzidine chromogen as a substrate. Sections were counterstained with hematoxylin.

Immunohistochemical results were evaluated by following uniform preestablished criteria. Immunoexpression was graded semiquantitatively (histoscore) by considering the percentage of cells stained (0%, 25%, 50%, 75%, and 100%) and intensity of the staining (negative staining, 0; light, 1; moderate, 2; and intense, 3). The maximum value was 300. Results of this histoscore were subjected to statistical analysis. Mean and SD were computed to define immunoexpression levels. The Mann–Whitney or Kruskal–Wallis test was conveniently used to assess the differences between sample types. A linear model was also used to adjust for potentially confounding variables (sex and age). A multivariate logistic regression model was fitted to determine a molecular-based profile associated with photoexposition. Sensitivity and specificity analysis was performed to assess its reproducibility, computing the area under the Receiver Operating Characteristic (ROC) curve. All analyses were performed using R statistical package, and the threshold for significance was set at 5% ($\alpha = 0.05$).

Other histopathological data were also analyzed as a second objective such as tumor differentiation (follicular, apocrine/eccrine or sebaceous) and BCC subtype to correlate with the CK expression. The criteria used in this work to diagnose adnexal differentiation in BCC were as follows[18] (see **Figure 1**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A58).

### Apocrine/Eccrine Differentiation

Decapitated cells, ducts (lined by cuboidal cells with an eosinophilic cuticular border or vacuolated alveolar units).

### Sebaceous Differentiation

Sebocytes and sebaceous ducts.

### Follicular Differentiation

Bulb and papilla (papillary mesenchymal bodies including circular-shaped papilla). Isthmus (cells with abundant pink cytoplasm that cornify into compact keratin without keratohyalin granules).

The degree of solar elastosis was evaluated by a pathologist, and those referred to as positive included from the mildest (presence of single-scattered elastotic fibers) to the most severe (clumps of elastotic fibers).

**TABLE 1.** Expression Pattern for the CK in Normal Skin and the Antibodies Used in the Immunohistochemistry[17]

| Antigen | Antibody-Clone | Dilution | Source | Skin Targets |
|---|---|---|---|---|
| CK5/6 | D5/16 B4 | Ready to use | DAKO, Glostrup, Denmark | Epidermis (basal and parabasal cells), hair follicle (all parts except the inner sheath), sebaceous gland (all parts), sweat glands (all parts except secretory cells) |
| CK7 | OV-TL 12/30 | Ready to use | DAKO, Glostrup, Denmark | Sebaceous gland (sebocytes and undifferentiated cells) and sweat glands (secretory cells) |
| CK14 | LL002 | 1:100 | Leica, Newcastle, United Kingdom | Epidermis (all layers except the corneum layer), hair follicle (all parts except the inner sheath), sebaceous gland (all parts), sweat glands (all parts except secretory cells) |
| CK15 | LHK15 | 1:100 | Leica, Newcastle, United Kingdom | Hair follicle (only in the bulge and follicular germinative cells), sebaceous gland (undifferentiated cells), and sweat glands (only in secretory cells) |
| CK17 | E3 | Ready to use | DAKO, Glostrup, Denmark | Hair follicle (isthmus and outer sheath) and sebaceous gland (only sebaceous ducts) |
| CK19 | RCK108 | Ready to use | DAKO, Glostrup, Denmark | Hair follicle (only in the bulge and follicular germinative cells) and sweat gland (only secretory cells) |

## RESULTS

The histoscore results with the expression of CK in each of the 3 anatomical locations are reflected in **Supplemental Digital Content 1** (see **Tables 1–3**, http://links.lww.com/AJDP/A59). CK5/6 expression showed significant differences depending on the anatomical BCC localization (adjusted $P = 0.0002$) (**Supplemental Digital Content 1**, **Table 4**, http://links.lww.com/AJDP/A60). Higher expression levels were detected in facial lesions, whereas in trunk samples, low CK5/6 were noted (Fig. 1), and nonphotoexposed samples exhibited an intermediate level of CK5/6 expression. CK7 expression showed also significant differences depending on the BCC localization (adjusted $P = 0.009$) (see **Table 4**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A60). Most BCC samples arising in nonphotoexposed areas showed high CK7 expression levels, whereas tumors located on the trunk and face (sun-exposed areas) exhibited negative or barely detectable levels (Fig. 1). In 5 of 30 facial samples, maximum levels of CK7 histoscore were detected, which suggests that different subsets of nodular facial BCCs regarding CK7 expression may exist (Figs. 2B, D). CK7 expression was also detected in all BCC located on the inner part of the labia majora (n = 4), a nonphotoexposed area devoid of hair follicles, and BCC arising in the nipple, another hairless area derived from a modified apocrine gland but located in an intermittent photoexposed area (Figs. 3B, D). In addition, on the axilla, a nonphotoexposed area where apocrine glands are abundant, the expression of CK7 was also highly positive (Figs. 2A, C). No statistically significant differences in the expression of CK14, CK15, CK17, and CK19 were detected among the different anatomical localizations.

To assess possible differences between sun-exposed and non–sun-exposed areas, all patients included in groups 1 and 2 (BCC from both chronic and intermittently photoexposed areas) were compared with patients with lesions arising in nonphotoexposed areas (group 3) (Table 2). No differences were detected regarding CK5/6 expression when comparing the degree of sun exposure. Only significant differences for the CK7 histoscore were detected (adjusted $P = 0.002$) in which photoexposed areas showed a histoscore level of 86.06 ($\pm$101.63), whereas non–sun-exposed areas showed 181.67 ($\pm$104.57). Moreover, because the solar elastosis degree is

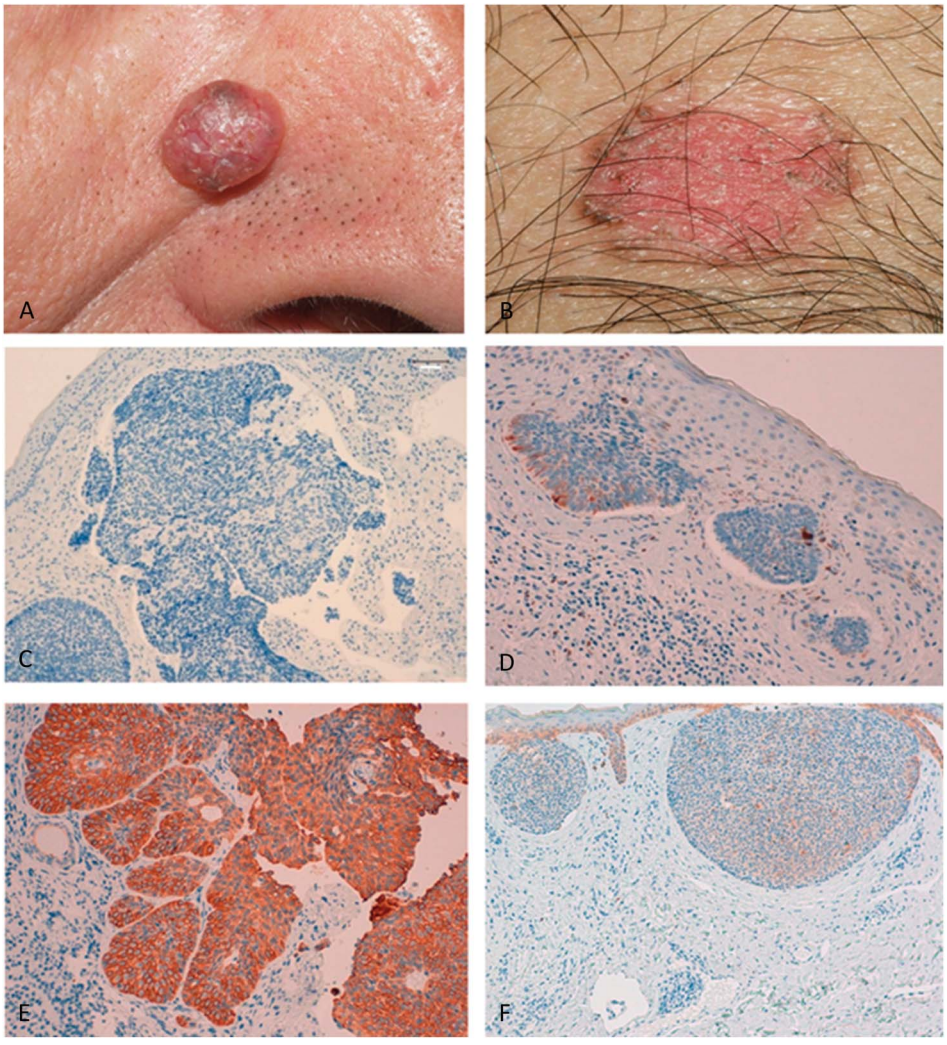


**FIGURE 1.** Representative images of nodular and superficial BCCs. A, Clinical image of a facial nodular BCC. B, Clinical image of a superficial BCC from the trunk (C) nodular BCC with negative CK7 expression. D, Superficial BCC with low CK7 expression. E, Nodular BCC with high CK5/6 expression. F, Superficial BCC with low CK5/6 expression. Original magnification: (C) ×100; (D) ×40; (E) ×100; (F) ×40.

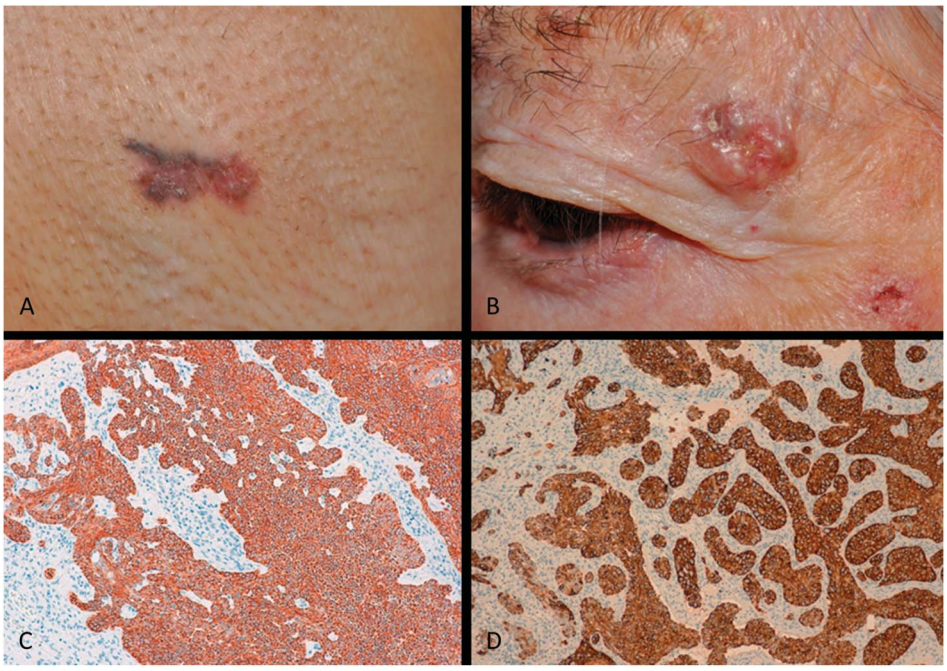


**FIGURE 2.** A, Nonphotoexposed BCC on the axilla showing intense expression of CK7 (C). B, Nodular BCC on the face with a very intense CK7 expression (D). Original magnification: (C) ×40; (D) ×100.

an additional manner to measure sun exposure, as expected, we have observed the same results while comparing CK expression with either the elastosis degree or anatomical location (Table 2 and see **Table 5**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A61). However, to assess whether the data obtained in our CK profile were reliable enough in the degree of photoexposure, we generated an ROC curve. We achieved an area under the curve of a 95% (AUC 0.95), which indicates that the CK profile correlates with the localization of BCCs in sun-exposed and non–sun-exposed areas (Fig. 4).

The secondary analysis of sex and age did not provide relevant information (see **Table 6**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A62). As expected, a relationship between those factors and the CK expression was not detected, except for CK14. Intriguingly, CK14 shows a significant *P* value when compared with sex. The mean histoscore of CK14 is slightly higher in women, but this finding needs further investigation.

We have also assessed the correlation of keratins with the histological BCC subtype irrespective of the anatomical location. We have observed a significant association of the

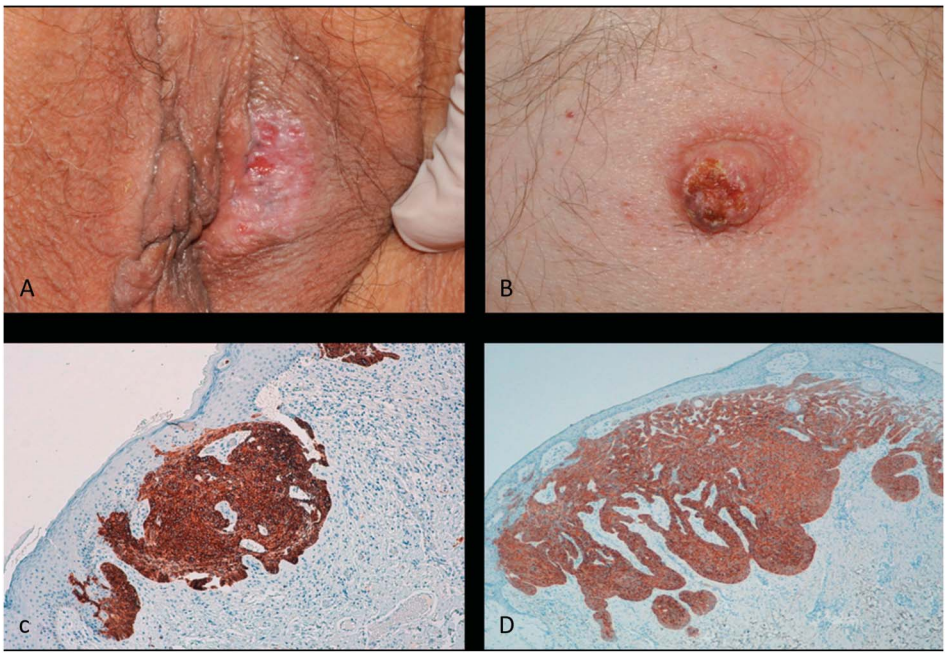


**FIGURE 3.** Nonphotoexposed BCCs showing high CK7 expression. A, Vulvar BCC showing an intensive CK7 expression (C). B, Ulcerated BCC from the nipple with high CK7 expression (D). Original magnification: (C) ×100; (D) ×40.

**TABLE 2.** Keratin Immunoexpression With Relation to Sun Exposure (Photoexposed vs. Nonphotoexposed)

| | Totals, n = 67 (100%) | Photoexposed, n = 52 (77.61%) | Nonphotoexposed, n = 15 (22.39%) | *P* | Adjusted *P* |
|---|---|---|---|---|---|
| CK7 | 107.46 (109.15) | 86.06 (101.63) | 181.67 (104.57) | 0.001 | 0.002 |
| CK5/6 | 158.21 (68.17) | 160.58 (62.10) | 150.00 (88.14) | 0.58 | 0.60 |
| CK19 | 47.39 (66.02) | 41.83 (61.58) | 66.67 (78.87) | 0.11 | 0.20 |
| CK14 | 215.67 (51.65) | 219.23 (39.80) | 203.33 (81.21) | 0.51 | 0.28 |
| CK17 | 209.33 (65.83) | 212.98 (62.72) | 196.67 (76.69) | 0.44 | 0.40 |
| CK15 | 24.22 (42.72) | 19.00 (38.98) | 42.86 (51.36) | 0.08 | 0.06 |

Mean (and SD) presented for each marker, assessing the differences with a Mann–Whitney. *P* values are adjusted by sex and age obtained with an analysis of variance test.

expression of CK5/6 and the histological subtype. The mean histoscore of this CK distinctly increases in nodular BCC compared with superficial ones (Table 3).

Finally, we have evaluated the association of CK expression with the pattern of adnexal differentiation. The samples were analyzed by the pathologist, and 3 groups of differentiation were defined: follicular (11 samples), apocrine/eccrine (11 samples), and sebaceous (3 samples). In total, 37% of the samples showed adnexal differentiation. Our data show that CK7 is highly expressed in BCC with apocrine/eccrine adnexal differentiation (see **Table 7**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A63). However, because of the low number of samples, statistical analyses are not reliable (ie, sebaceous differentiation).

## DISCUSSION

Stem cells from the outer sheath of the hair follicle or hair bulge are the most widely accepted theory on the cellular origin of BCC. The histological similarities between some clear-cut follicular tumors and BCC along with the low frequency of BCC arising in hairless areas[19,20] and the development of BCC on follicular stem cell CK15+[14] seem to support this hypothesis. A possible origin from the interfollicular epithelium has also been suggested, based on studies in murine models.[3,4,21]

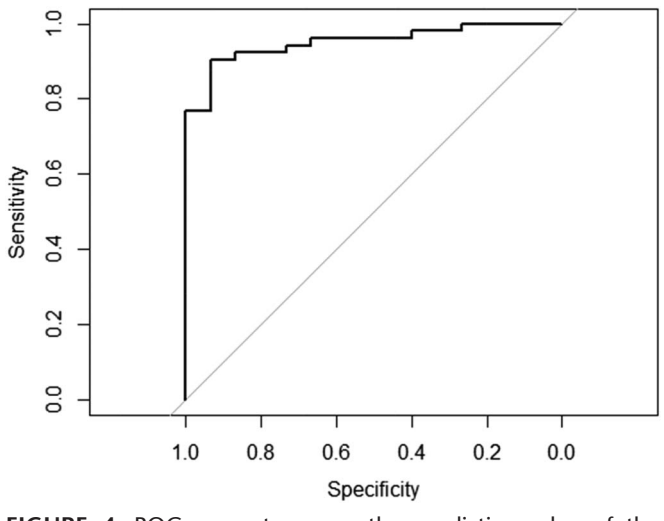


**FIGURE 4.** ROC curve to assess the predictive value of the multivariate logistic regression model to discriminate the samples corresponding to the photoexposed versus nonphotoexposed site. Estimation of the area under the curve (AUC) = 0.95. This mathematical model allows us to predict correctly where a BCC is located, photoexposed versus nonphotoexposed site, according to the CK profile (CK5/6, CK7, CK14, CK17, and CK19) in the 95% of all the cases.

The underlying molecular alterations in BCC have been studied extensively. In Gorlin syndrome, an autosomal dominant genetic disorder characterized by the early development of multiple BCCs, a constitutive mutation of the *sonic hedgehog* pathway, alongside loss of the inhibitory function of the membrane receptor, PTCH, has been seen. In sporadic BCC, the sonic hedgehog pathway seems to be altered in 40%–60% of cases: 20%–40% as a result of a double mutation in the *PTCH1* tumor suppressor gene and in 10%–20% of patients because of a single mutation of the SMO oncogene.[5–8] Ultraviolet (UV) radiation may induce deleterious mutations in one or both alleles giving rise to pathway activation. In rare instances, alterations of the Wnt pathway and the involvement of β-catenin or the ERK (MAP kinase) pathway have been found.[22,23]

Not exceptionally, BCC may arise in non–sun-exposed areas, such as the axilla or pubic region, and the etiopathogenic role of UV exposure in those areas could not be responsible. Additionally, in rare instances, BCC is observed to develop in hairless areas such as the vulvar semimucosa or the nipple, resulting in some controversies regarding its unequivocal follicular origin. The CK profile has been used to establish the tissue origin of the tumor because it is commonly conserved during cancerous transformation. However, it is important to take into account different aspects when interpreting CK data: the degree of tumor differentiation, the specificity and reliability of antibodies, and also the tumor heterogeneity. To ascertain the real niche involved in the origin of the neoplasia, the CK profile may be used in conjunction with other features such as morphological data.[24] In this work, the CK profile we found suggests a glandular origin of BCC arising in nonhairy and non–sun-exposed areas, but our data cannot exclude the other possible hypothesis. We have compared CK expression patterns in a series of BCCs arising in areas chronically exposed to sunlight, BCCs developing in areas with acute and intermittent photoexposure, and BCCs arising in nonphotoexposed areas. To our knowledge, no previous studies comparing CK expression in BCC with the anatomical location or the degree of exposure to sunlight have been published.

**TABLE 3.** Association of the BCC Subtype Group With CKs

| | | BCC Subtypes | | | |
|---|---|---|---|---|---|
| | **Totals** | **Infiltrative** | **Nodular** | **Superficial** | ***P*** |
| CK7 | 107.46 (109.15) | 141.67 (137.69) | 103.68 (120.94) | 108.33 (94.99) | 0.46 |
| CK5/6 | 158.21 (68.17) | 158.33 (72.17) | 196.32 (46.11) | 115.00 (64.53) | <0.00001 |
| CK19 | 47.39 (66.02) | 50.00 (50.00) | 46.32 (64.59) | 48.33 (70.69) | 0.89 |
| CK14 | 215.67 (51.65) | 233.33 (57.74) | 222.06 (51.04) | 206.67 (52.08) | 0.52 |
| CK17 | 209.33 (65.83) | 200.00 (0.00) | 219.12 (53.68) | 199.17 (79.73) | 0.81 |
| CK15 | 24.22 (42.72) | 58.33 (80.36) | 25.76 (48.20) | 18.75 (29.36) | 0.55 |

Mean (and SD) are shown. *P* values from a Mann–Whitney test shown to assess the relationship.

CK7, a protein mainly expressed in the secretory cells of sweat and sebaceous glands,[17] showed a significantly higher expression in anatomic areas not exposed to sunlight and specifically in apocrine gland-rich areas such as genital and axillary regions and the nipple. Expression of CK7 is usually observed in glandular epithelia such as mammary ductal, pancreatic, or urothelial epithelia. CK7 is expressed also in normal or tumor-derived apocrine and sebaceous glands[12,17] and is considered a marker of mammary and extramammary Paget disease.[25,26] The possibility that BCC-developing apocrine-rich areas such as the axilla and pubic areas may have a common origin in the stem cells of apocrine gland could be speculated. In fact, fewer than 100 cases of axillary BCCs have been published in the literature[27–29] (Fig. 2), but no studies evaluating the CK profile in such tumors have been reported. A similar situation could be postulated for the rare observation of BCC developing on the nipple,[30] an apocrine-rich area, devoid of hair follicles,[31] in which we have also observed an intense CK7 expression (Figs. 3B, D). Among other nonphotoexposed regions, in some cases of vulvar BCC developing on the inner part of the labia majora, another hairless region containing noncanonical sebaceous glands and no traces from apocrine glands, we have also observed high expression of CK7 (Figs. 3A, C), a feature that may also suggest a possible sebaceous stem cell[32] as the origin of these tumors.[33]

As expected, CK7 is also significantly associated with negative elastosis in agreement with its higher expression in non–sun-exposed areas (see **Table 5**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A61). However, in our series, 14 of 30 facial nodular BCCs were CK7 negative, whereas 5 of 30 showed an intense CK7 expression (histoscore 300) (see **Table 1**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A61). Alessi et al[12] analyzing different series of BCC tumors showed that only a subgroup of BCC, BCC with adnexal differentiation, exhibited high positivity of CK7 (88%), whereas in the other subgroups, CK7 positivity was only 42%. These authors postulated a possible glandular origin of some BCC. Consistently, we have observed apocrine/eccrine differentiation in non–sun-exposed samples and follicular differentiation in facial samples. Then, we can obtain a significant association of CK7 expression and apocrine/eccrine differentiation. This result is consistent with our proposal, but we believe that the compilation of additional samples (ie, sun-exposed BCC with eccrine or sebaceous differentiation) will be required in future works to confirm these results (see **Table 7**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A61).

In our study, we have been unable to detect a correlation between CK7 and CK19 expression levels. This feature has been previously reported by other authors.[17] We have observed low levels of CK15, a potential marker of the stem cells of the hair bulge,[14] in all the BCCs studied. Similar results have been described in previous studies.[14,17] However, histoscore levels of CK15 and CK19 were higher in nonphotoexposed regions, although that was not statistically significant (Table 2). Both CKs are expressed in the secretory cells of sweat glands[17] and in the bulge of the hair follicle. As reported in other studies, CK17 was positive in all the BCCs studied.[12,15,34–36] CK17 is a marker of follicular differentiation because it is weakly positive in the upper portion of the outer root sheath of the hair follicle and is also expressed in the deep portion. CK14 was also positive in all the BCCs studied.[15,34–37] CK14 was expressed primarily in the subinfundibular outer sheath region of the hair follicle.[12] No significant differences among the evaluated groups were detected regarding the expression of this CK.

We have studied, as a secondary objective, whether some differences are detected in the CK profile in the different BCC subtypes (nodular BCC, superficial BCC, and infiltrative BCC), and we obtained differences in CK5/6 expression in nodular BCC. Facial BCC showed high levels of CK5/6, and this association is not detected when comparing sun-exposed and non–sun-exposed BCC (Table 2). Because we have obtained almost all nodular BCCs from the face and most of the superficial ones from the trunk, the same significant association is observed with the expression of CK5/6 and the anatomical location (see **Table 4**, **Supplemental Digital Content 1**, http://links.lww.com/AJDP/A61). From our data, we cannot, therefore, conclude whether the association is with either the anatomical localization or the histological subtype. However, Alessi et al 2008[12] did not find any association of CK5/6 expression with the histological subtype. Taking these data into account, the possibility of a different expression of CK5/6 depending on the anatomical localization, face (chronic sun-exposed) versus trunk (discontinuous sun-exposed), is open.

In conclusion, according to the expression of CK7 and its correlation with BCCs located in nonphotoexposed areas, we suggest that those BCCs are derived from simple glandular epithelia. In agreement with this, nonphotoexposed areas such as the axilla and pubis or in some special locations such as the

nipple or the vulvar semimucosa are rich in apocrine and sebaceous glands. In such instances, etiopathogenic factors other than ultraviolet light exposure could be responsible.

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