

UC Irvine

UC Irvine Previously Published Works

Title

Cutaneous Lymphadenoma Is a Distinct Trichoblastoma-like Lymphoepithelial Tumor With Diffuse Androgen Receptor Immunoreactivity, Notch1 Ligand in Reed-Sternberg-like Cells, and Common EGFR Somatic Mutations

Permalink

<https://escholarship.org/uc/item/7hz7m9hg>

Journal

The American Journal of Surgical Pathology, 45(10)

ISSN

0147-5185

Authors

Monteagudo, Carlos
Fúnez, Rafael
Sánchez-Sendra, Beatriz
et al.

Publication Date

2021-10-01

DOI

10.1097/pas.0000000000001727

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Cutaneous Lymphadenoma Is a Distinct Trichoblastoma-like Lymphoepithelial Tumor With Diffuse Androgen Receptor Immunoreactivity, Notch1 Ligand in Reed-Sternberg-like Cells, and Common EGFR Somatic Mutations

Carlos Monteagudo, MD, PhD,*† Rafael Fúnez, MD,‡ Beatriz Sánchez-Sendra, BSBC,† José F. González-Muñoz, BBiot,* Gema Nieto, BSB,† Clara Alfaro-Cervelló, MD, PhD,* Amelia Murgui, MD, PhD,§ and Ronald J. Barr, MD||

Abstract: The term “cutaneous lymphadenoma” was coined in this journal for an unusual lymphoepithelial cutaneous adnexal neoplasm, possibly with immature pilosebaceous differentiation. Some authors further proposed that cutaneous lymphadenoma was an adamantinoid trichoblastoma. However, although a hair follicle differentiation is widely accepted, the fact that this is a lymphoepithelial tumor is not appropriately explained by the trichoblastoma hypothesis. Our goal was to further clarify the phenotypic and genotypic features of cutaneous lymphadenoma in a series of 11 cases. Histologically, a lobular architecture surrounded by a dense fibrous stroma was present in all cases. The lobules were composed of epithelial cells admixed with small lymphocytes and isolated or clustered large Reed-Sternberg-like (RS-L) cells. The epithelial cells were diffusely positive for the hair follicle stem cell markers CK15, PHLDA1, and for androgen receptor. No immunostaining for markers of sebaceous differentiation was found. Intraepithelial lymphocytes were predominantly CD3⁺, CD4⁺, FoxP3⁺ T cells. RS-L cells showed both strong Jagged-1 and Notch1 cytoplasmic immunostaining. Androgen-regulated NKX3.1 nuclear immunostaining was present in a subset of large intralobular cells in all cases.

Double immunostaining showed coexpression of NKX3.1 and CD30 in a subset of RS-L cells. No immunostaining for lymphocytic or epithelial markers was present in RS-L cells. EGFR, PIK3CA, and FGFR3 somatic mutations were found by next-generation sequencing in 56% of the cases. We consider that cutaneous lymphadenoma is a distinct benign lymphoepithelial tumor with androgen receptor and hair follicle bulge stem cell marker expression, RS-L cell-derived Notch1 ligand, and common EGFR gene mutations.

Key Words: cutaneous lymphadenoma, adamantinoid trichoblastoma, androgen receptor, Notch1, Jagged-1, EGFR

(*Am J Surg Pathol* 2021;45:1382–1390)

In 1991, Santa Cruz et al¹ published in this journal, under the label “cutaneous lymphadenoma,” the seminal description of an unusual and previously unrecognized lymphoepithelial cutaneous tumor which the authors interpreted as an adnexal neoplasm, possibly with immature pilosebaceous differentiation.¹ Cutaneous lymphadenoma is characterized by lobules of basaloid cells with peripheral palisading and a dense fibrous stroma. Numerous small lymphocytes are present within the lobules and a limited number are found in the stroma.^{1–7} Intralobular Langerhans’ cells are usually numerous. An outstanding feature is the presence of large Reed-Sternberg-like (RS-L) cells with amphophilic cytoplasm, vesicular nuclei, and prominent nucleoli.^{1–7} A few years later, other authors proposed that cutaneous lymphadenoma was a variant of trichoblastoma for which they proposed the term “adamantinoid trichoblastoma.”^{8–11}

Although some authors have proposed that cutaneous lymphadenomas are trichoblastomas with regressive features,¹² the pathogenesis of aberrant lymphoepithelial interactions in cutaneous lymphadenoma^{1,4,6} represents a challenge as this tumor is characterized by epithelial cells with hair follicle bulge stem cell marker expression and it is well known that the hair follicle bulge region is a site of

From the *Department of Pathology, University Clinic Hospital, INCLIVA Biomedical Research Institute; Departments of †Pathology; §Biochemistry and Molecular Biology, University of Valencia, Valencia; ‡Department of Pathology, Costa del Sol Health Agency, Málaga, Spain; and ||Barr Dermatopathology, Laguna Beach, CA.

Conflicts of Interest and Source of Funding: Supported by grant PI17-02019 from Instituto de Salud Carlos III, Spain; and FEDER European funds. No funding received from National Institutes of Health, Wellcome Trust or Howard Hughes Medical Institute. The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Carlos Monteagudo, MD, PhD, Department of Pathology, University Clinic Hospital, Avenida Blasco Ibáñez, 15, Valencia 46010, Spain (e-mail: carlos.monteagudo@uv.es).

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

TABLE 1. The Antibodies Used in the Immunohistochemical Study

Antibody	Clone	Source	Dilution	Retrieval
CK15	LKH15	Diagnostic Biosystems	1/100	PTLINK high pH
CK7	OV-TL-12/30	Agilent Dako	Prediluted	PTLINK high pH
CK	AE1-AE3	Agilent Dako	Prediluted	PTLINK high pH
PHLDA1	—	Sigma-Aldrich	1/50	PTLINK high pH
AR	AR441	Agilent Dako	1/100	PTLINK high pH
CD1a	010	Agilent Dako	Prediluted	PTLINK high pH
Langerin	12D6	Gennova	1/50	PTLINK high pH
CD45	2B11+PD7/26	Agilent Dako	Prediluted	PTLINK high pH
CD3	—	Agilent Dako	Prediluted	PTLINK high pH
CD4	4B12	Agilent Dako	Prediluted	PTLINK high pH
CD8	C8/144B	Agilent Dako	Prediluted	PTLINK high pH
CD20	L26	Agilent Dako	Prediluted	PTLINK high pH
CD30	BER-H2	Agilent Dako	Prediluted	PTLINK high pH
FoxP3	—	Abcam	1/150	PTLINK high pH
Adipophilin	—	Cell Marque	Prediluted	PTLINK high pH
EMA	E29	Agilent Dako	Prediluted	PTLINK high pH
Factor XIIIa	AC-1A1	Gennova	1/100	PTLINK high pH
Beta-catenin	Beta-catenin 1	Agilent Dako	Prediluted	PTLINK high pH
NKX3.1	—	Gennova	1/100	PTLINK high pH
Jagged-1	—	Santa Cruz	1/100	PTLINK high pH
Notch1	—	Abcam	1/200	PTLINK high pH
CD34	QBEnd 10	Agilent Dako	Prediluted	PTLINK high pH
CD163	10D6	Biocare Medical	1/100	PTLINK high pH
CD68	KP1	Agilent Dako	Prediluted	PTLINK high pH

FoxP3 indicates forkhead box P3.

immune privilege.¹³ Moreover, specific markers of this tumor that would facilitate its differential diagnosis with other benign and malignant cutaneous neoplasms are still lacking.

Our goal was to analyze the immunophenotypic and genetic features of cutaneous lymphadenoma in a series of 11 unpublished cases in an attempt to find useful markers for this unusual tumor that may improve its differential diagnosis with other cutaneous adnexal tumors.

MATERIALS AND METHODS

After institutional review board approval, 11 cases of cutaneous lymphadenoma were retrieved from the authors' institutional archives. These cases had been collected during the period from January 1, 2000, to December 31, 2019. None of these cases have been reported previously. These lesions were reviewed by 3 pathologists (C.M., R.J.B., and R.F.) following the histologic criteria from the original description.¹

Immunohistochemistry

The immunohistochemical study was performed on 4- μ m-thick sections from formalin-fixed paraffin-embedded tissue with the antibodies and dilutions detailed in Table 1.

Next-generation Sequencing

Next-generation sequencing was performed on formalin-fixed paraffin-embedded tissue sections from 9 cases of cutaneous lymphadenoma. DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, CA) and RNA was extracted using the Rneasy FFPE Kit (Qiagen). Concentrations of DNA and RNA were determined by fluorometric quantitation using Qubit 2.0

Fluorometer (Thermo Fisher Scientific, Waltham, MA) with Qubit DNA dsDNA BR Assay, Qubit DNA 1 \times dsDNA HS Assay, Qubit RNA BR Assay and Qubit RNA HS Assay. RNA was reverse transcribed before library preparation using SuperScript VILO cDNA Synthesis Kit (Thermo Fisher Scientific).

Starting from 10 ng DNA and RNA, samples were evaluated by the Oncomine Focus Assay (Thermo Fisher Scientific) in an Ion 520 Chip. Preparation of independent libraries for DNA and RNA, and the template were performed by the automated Oncomine Chef System (Thermo Fisher Scientific).

Sequencing was analyzed with Ion Reporter, version 5.14 (Thermo Fisher Scientific). Variants were annotated using Oncomine Focus Assay Annotations, v1.4 r0 (Thermo Fisher Scientific) and were expressed according to the hg19 reference genome (GRCh37; Genome Reference Consortium Human Build 37; NCBI, Bethesda, MD).

RESULTS

Clinical data are summarized in Table 2. The most common clinical appearance was that of a long-standing papule or nodule, ≤ 10 mm in diameter, commonly located on the head and neck and, particularly, on the face.

The most distinctive histopathologic features were the presence of dermal lobules, nests, and/or cords of epithelial cells that were only occasionally connected to the epidermis or adnexal epithelium, along with the presence of numerous intraepithelial lymphocytes (Fig. 1). Some of the lobules and nests had peripheral palisading with columnar or cuboidal cells (Fig. 1C). In all cases, isolated large pale and/or stellate cells with one to several large

TABLE 2. Clinical Features

Case #	Sex	Age (y)	Location	Clinical Features
1	Female	78	Temple	Papule
2	Male	38	Upper lip	Nodule
3	Female	50	Forehead	Nodule
4	Male	30	Upper lip	Papule
5	Female	42	Glabella	Papule
6	Male	63	Superciliary	Papule
7	Male	49	Naso labial	Papule
8	Female	—	Forehead	Papule
9	Female	55	Front	Papule
10	Female	61	Thigh	Nodule
11	Female	34	Preauricular	Papule

nuclei and prominent central nucleoli giving them a RS-L appearance were also found within some of the epithelial lobules (Figs. 1C, D). Mitotic figures were scarce in the epithelial component and only occasionally found in large RS-L cells. The epithelial nests were usually surrounded by the desmoplastic stroma (Fig. 1B). When lymphocytes were present in the perilobular stroma these were usually fewer than those in the intralobular compartment.

Immunohistochemistry

The immunohistochemical findings are summarized in Table 3.

The epithelial cell population of the lobules and nests was diffusely positive for CK15, plectstrin homology like domain family A member 1 (PHLDA1) and androgen receptors (ARs) in all cases (Fig. 2), but while CK15 and PHLDA1 immunostaining were also present in cells that exhibit peripheral palisading (Fig. 2B), AR was constantly negative in cuboidal or columnar peripheral cells (Fig. 2D). Large RS-L cells were negative for CK15 (Fig. 2B), PHLDA1, and AR (Fig. 2D). No immunostaining for adipophilin, factor XIIIa, and epithelial membrane antigen (EMA) was found in the intralobular epithelial population. CK7 was only focally positive in 2 cases.

Intraepithelial lymphocytes were predominantly CD3⁺, with a higher number of CD4 than CD8, and the majority being forkhead box P3 (FoxP3)-positive (Fig. 3). A dense intralobular network of intraepithelial dendritic CD1a⁺, Langerin⁺ Langerhans' cells was commonly found. A limited number of macrophages were present, with a predominance of CD68 in the intralobular compartment, whereas CD163 was overrepresented in the interlobular stroma.

Membranous CD30 immunoreactivity was present in a subset of intralobular large RS-L cells (Fig. 4A), but no immunostaining for CD45, CD3, CD4, CD8, or CD20 was found in this cell population. EMA immunoreactivity was only present in few isolated cells within the tumor

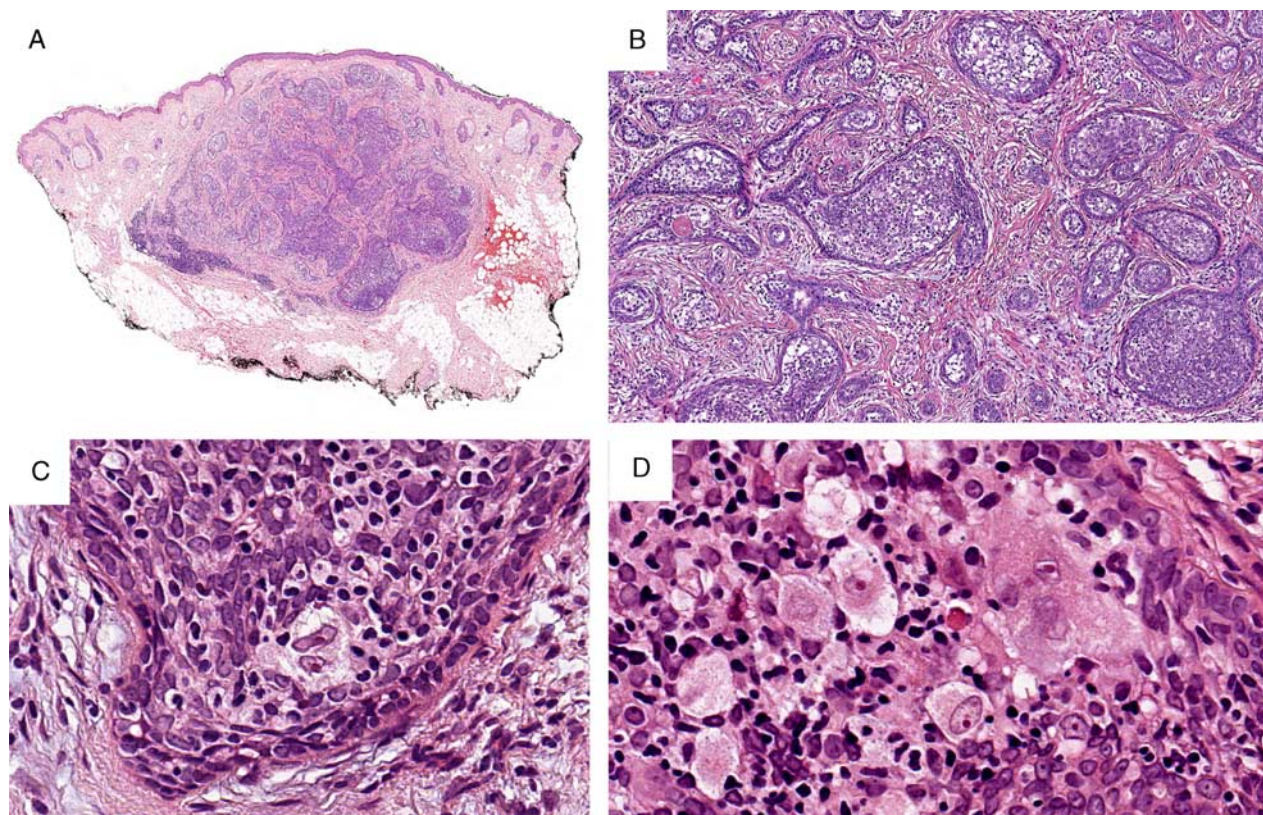


FIGURE 1. Histopathologic features of cutaneous lymphadenoma. A, Scanning view of the lobulated dermal growth. B, Lymphoepithelial tumor nests surrounded by desmoplastic stroma. C, Tumor nest with a RS-L cell and peripheral cuboidal cells. D, Large and RS-L cells within the tumor lobules.

TABLE 3. Immunohistochemical Findings

Antibody	Immunostaining		
	EC	RS-L Cells	L
CK15	+++	–	–
CK7	–	–	–
AE1-AE3	+++	–	–
PHLDA1	+++	–	–
AR	+++	–	–
CD45	–	–	+++
CD3	–	–	++
CD4	–	–	++
CD8	–	–	+
CD20	–	–	+
CD30	–	+	–
FoxP3	–	–	++
Adipophilin	–	–	–
EMA	–	–	–
Factor XIIIa	–	–	–
Beta-catenin*	–	–	–
NKX3.1	–	+	–
Jagged-1	–	+++	+
Notch1	+	+	–
CD34	–	–	–
CD163	–	–	–
CD68	–	–	–

Immunohistochemical score: negative (–), <5% of positive cells; +, 5% to 25% positive cells; ++, 26% to 50% positive cells; +++, 51% to 100% positive cells.

*Only nuclear immunostaining for beta-catenin was considered.

EC indicates epithelial cells; FoxP3, forkhead box P3; L, lymphocytes.

lobules in 1 case. Interestingly, although RS-L cells were negative for AR (Fig. 2D), nuclear immunostaining for the AR-regulated NK3 homeobox 1 (NKX3-1) was present in some RS-L cells in all cases. Double immunohistochemistry showed coexpression of NKX3.1 and CD30 in some RS-L (Fig. 4A), but CD30⁺/NKX3.1[–], and CD30[–]/NKX3.1⁺ cells were also present. In a few peripheral sebocytes of normal adjacent sebaceous glands, NKX3.1 immunostaining was also found (Fig. 4B).

Notch1 cytoplasmic immunostaining was present in RS-L cells and in the epithelial stem cell population, being much higher in the former (Fig. 4C). Moreover, RS-L cells showed a strong cytoplasmic Jagged-1 immunostaining (Fig. 4D) whereas no significant Jagged-1 immunoreactivity was found in intralobular T cells. Coexpression of Jagged-1 and NKX3.1 was found in RS-L cells (Fig. 4D).

Next-generation Sequencing

Next-generation sequencing analysis showed that 5 of the 9 analyzed cases of cutaneous lymphadenoma (56%) presented gain-of-function missense EGFR, FGFR3, and PIK3CA pathogenic somatic mutations (Table 4). In addition, in 2 of the 9 cases (22%), ALK, MTOR, ERB3, MET, and/or FGFR4 pathogenic mutations were found (Table 4). All epidermal growth factor receptor (EGFR) mutations are pathogenic¹⁴ and their variant allele frequencies ranged from 4% to 21% (Table 5). Two cases shared the same p.Asp761Asn EGFR mutation.

DISCUSSION

Cutaneous lymphadenoma is a lymphoepithelial cutaneous tumor of disputed histogenesis¹⁵ and benign clinical behavior.^{1,4,6} Other lymphoepithelial cutaneous lesions are heterotopic dermal thymus and lymphoepithelial-like carcinoma.^{4,6} Although Kazakov et al¹⁶ reported 1 case of cutaneous lymphadenoma with a lymphoepithelial-like carcinoma component which metastasized to regional lymph nodes, the former is distinct and easily distinguished from the latter.⁶

With regard to the statement by the authors of the seminal description that cutaneous lymphadenoma has definite resemblance to desmoplastic trichoepithelioma and might represent a close entity,¹ several authors considered cutaneous lymphadenoma as an adamantinoid trichoblastoma and stated that “desmoplastic trichoepithelioma and adamantinoid trichoblastoma are merely different histopathologic expressions of a distinctive benign neoplasm composed of follicular germinative cells.”^{8–11}

Further studies reported that, similar to trichoblastoma, cutaneous lymphadenoma is positive for CK15 and PHLDA1, which are markers of hair follicle bulge stem cells.^{17–19} However, as trichoblastoma and desmoplastic trichoepitheliomas are AR negative^{17,20–23} our findings of a diffuse strong AR immunostaining in cutaneous lymphadenoma argues against considering it as a trichoblastoma. In fact, AR is one of the immunohistochemical markers proposed for the differential diagnosis between trichoblastoma and basal cell carcinoma (BCC), the latter showing commonly focal AR immunoreactivity.¹⁸ It is interesting to note that fibroepithelioma of Pinkus usually shows focal AR immunostaining,²⁴ while PHLDA1 is only positive in thin epithelial strands and not in basaloid nests, which some authors consider to be the BCC component.²⁵ Therefore, despite the fact that cutaneous lymphadenoma has similar histologic features similar to desmoplastic trichoepithelioma, our finding of strong and diffuse AR immunostaining in cutaneous lymphadenoma proves that the 2 are distinct tumors. Moreover, the invariable presence of intraepithelial lymphocytes and RS-L cells in cutaneous lymphadenoma, but not in trichoblastomas, also supports this contention. Furthermore, the diffuse pattern of AR immunostaining in cutaneous lymphadenoma is also different from the focal pattern (usually <5% of positive cells) found in BCC.²³

In normal pilosebaceous units, AR is generally expressed in the dermal papilla and sebaceous glands, but its expression in the follicular epithelium is controversial.²⁶ Although most authors have not found AR expression in the outer root sheath, hair bulb and bulge,^{26,27} Bayer-Garner et al²⁸ reported AR immunoreactivity in hair follicle basal cells adjacent to the acrotrichium, and Kretzchmar et al²⁹ found AR nuclear immunostaining in mice in the dermal papillae and also in the adjacent hair bulb during the telogen, catagen, and early anagen phases, but not during full anagen. AR mRNA has been detected in the inner and outer root sheath of male and female sexual hairs.³⁰ With regard to our finding that no nuclear β -catenin immunostaining was found in cutaneous lymphadenoma, Kretzchmar et al²⁹ found that

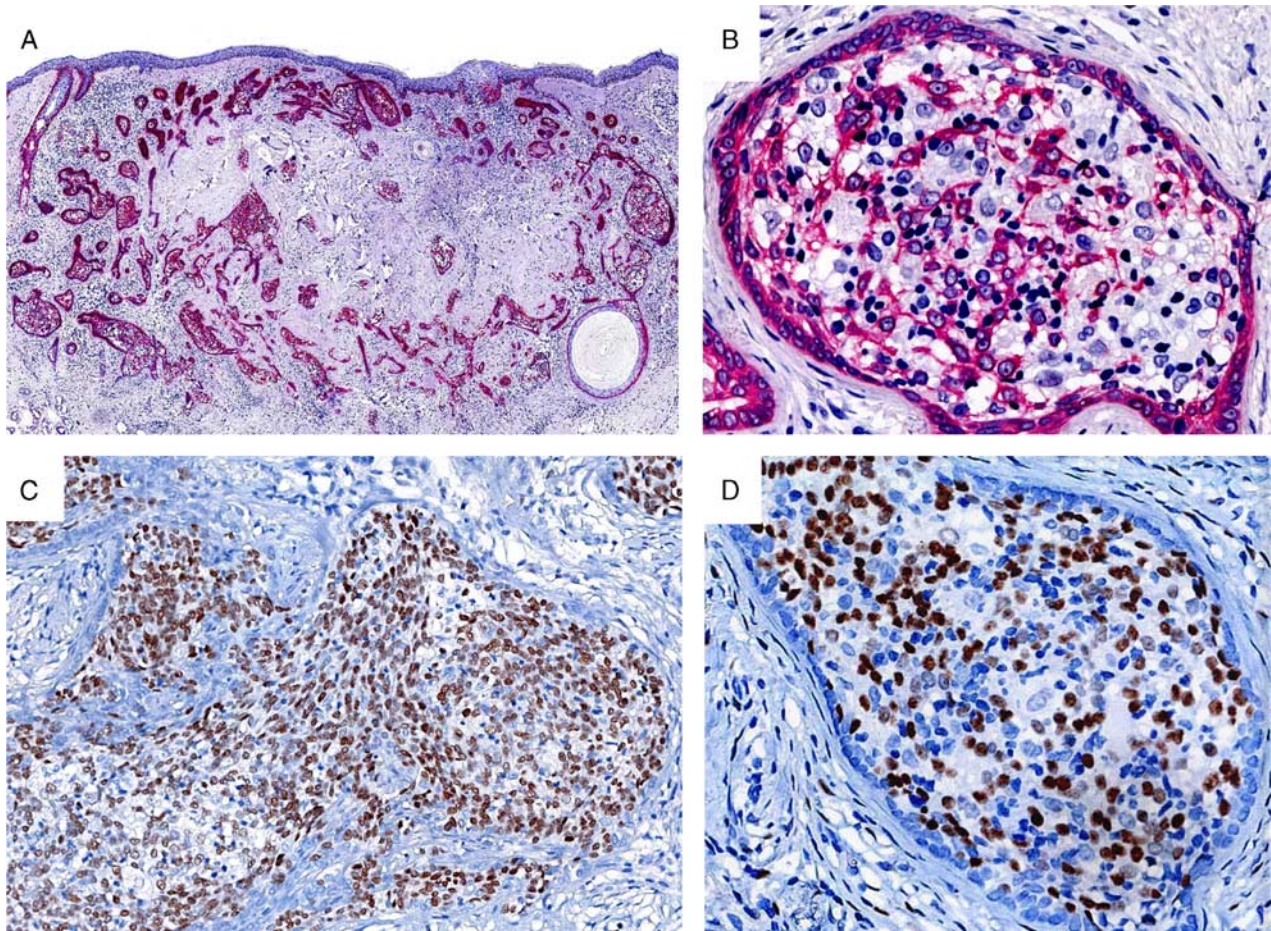


FIGURE 2. A and B, CK15 immunostaining. Low-power view showing a strong diffuse immunostaining in epithelial cells (A), no immunoreactivity can be found in the large cell component (B). C and D, AR immunostaining. Most epithelial cells show nuclear immunoreactivity (C), with the exceptions of peripheral cells and the large cell population (D).

AR and β -catenin display a reciprocal pattern of expression, by which, when nuclear AR is present then nuclear β -catenin is absent and vice versa. Therefore, these authors proposed that AR acts as a negative regulator of β -catenin/Wnt-dependent transcription.²⁹ As we have shown that all cases of cutaneous lymphadenoma show diffuse and strong nuclear AR expression in the hair follicle epithelial stem cell component, this feature explains the absence of nuclear β -catenin immunostaining.

NKX3.1 is an androgen-regulated homeobox protein commonly expressed in prostate carcinomas,³¹ but which has not been reported in the normal skin or in cutaneous neoplasms.³² The strong AR expression in cutaneous lymphadenoma led us to check NKX3.1. We found a selective nuclear immunostaining in a subset of large intralobular RS-L cells. Only some of these cells coexpressed CD30, whereas none coexpressed CD45, CD3, CD4, CD8, or cytokeratins. Interestingly, in normal adjacent skin, NKX3.1 immunostaining was found in some peripheral sebocytes, an observation not previously reported. This finding together with that of diffuse AR immunostaining might support incomplete sebaceous differentiation of cutaneous lymphadenoma as

suggested in the original description.¹ However, no immunoreexpression of adipophilin, factor XIIIa and/or EMA, which are currently considered the most sensitive and/or specific markers for sebaceous differentiation, was found, making this hypothesis highly unlikely.

The characteristic presence of numerous lymphocytes within the epithelial lobules was initially interpreted as aberrant lymphoepithelial trafficking or interaction.^{1,3,4} However, Magro's group has recently proposed that cutaneous lymphadenoma should be considered trichoblastomas with regressive features.¹² Nevertheless, in our opinion, the absence of clinical evidence of inflammation, the invariable presence, even in early stages, of intraepithelial T lymphocytes, including numerous regulatory T cells (Tregs), and the constant finding of intraepithelial large RS-L cells, argue against a degenerative regressing phenomenon. Fujimura et al³³ hypothesized that lymphocytes in cutaneous lymphadenoma may play roles in tuning and maintaining the homeostasis of this tumor. In agreement with this interpretation, current knowledge supports the role of regulatory T lymphocytes in hair follicle growth.³⁴ Indeed, although the hair follicle bulge is an area

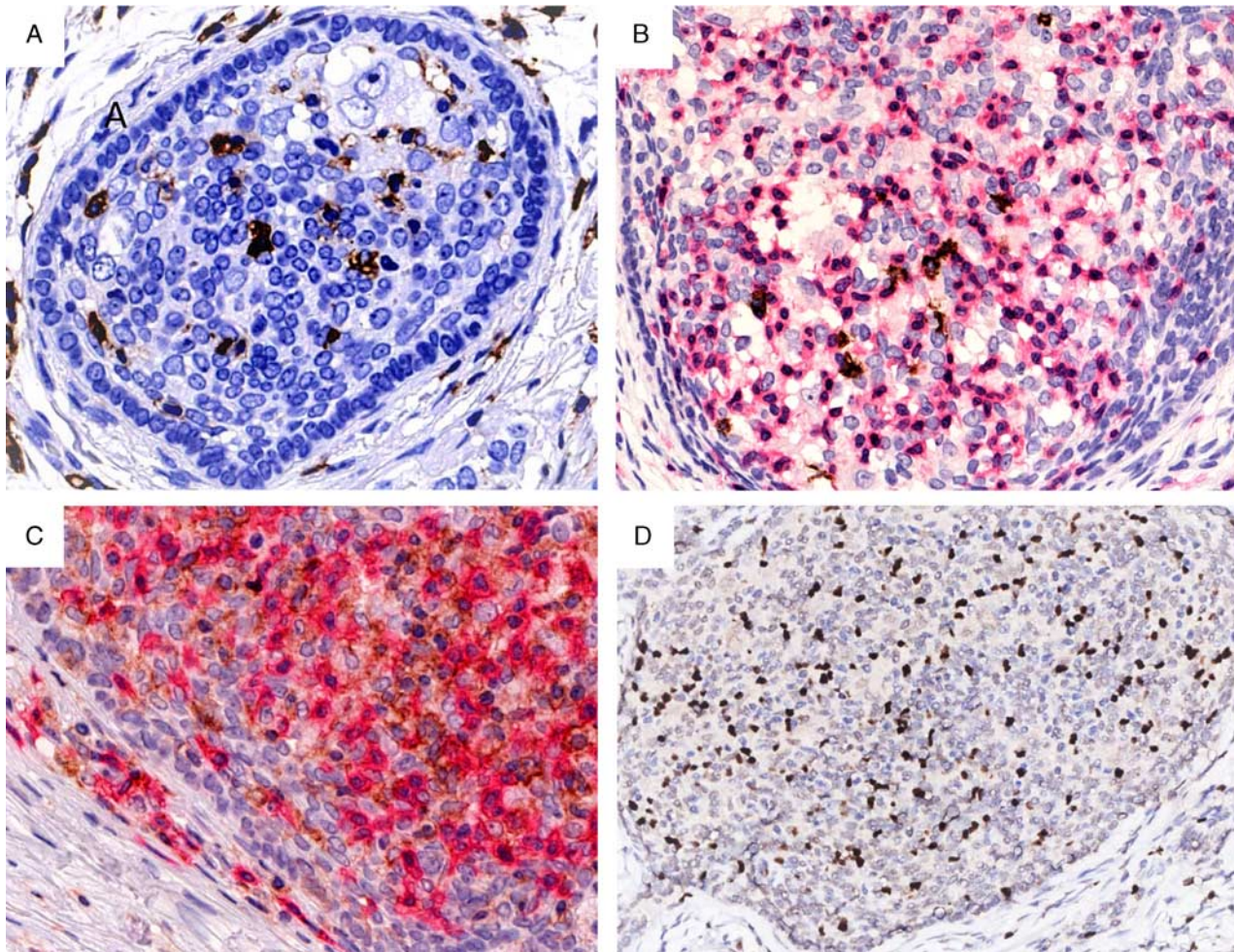


FIGURE 3. Immunoreactivity of intralobular lymphocytes. CD45 is positive in lymphocytes, but not in RS-L cells (A). CD3 (B—magenta) is positive in most lymphocytes, whereas CD20 is only positive in a minor lymphocytic population (B—brown). CD4 immunostaining is present in most lymphocytes (C—magenta) whereas CD8 is minority (C—brown). Forkhead box P3 immunoreactivity in numerous intralobular lymphocytes (D) (A and D, Immunoperoxidase with DAB chromogen; B, double immunohistochemistry with CD3-magenta chromogen and CD20-DAB chromogen; C, double immunohistochemistry with CD4-magenta chromogen and CD8-DAB chromogen).

of relative immune privilege,^{13,35} it is currently accepted that mature hair follicles have a distinctive immune system.³⁵ Hair follicles are constantly interacting with intraepithelial T lymphocytes and Langerhans cells as well as macrophages and mast cells located in the connective tissue sheath.^{35,36} In fact, accumulation of Langerhans cell is associated with Tregs expansion.³⁷ Hair follicle stem cells are essential for cyclic hair follicle growth (anagen), regression (catagen), and quiescence (telogen).³⁸ Hair follicle entry into the anagen phase requires the activation of hair follicle stem cells that expand to give rise to a new anagen hair follicle.³⁵ Ali et al³⁴ demonstrated that suppression of inflammation is not the major mechanism by which Tregs promote hair follicle stem cell proliferation and differentiation. Tregs promote hair follicle regeneration by augmenting hair follicle stem cells proliferation and differentiation.³⁴ Accumulation of activated Treg cells in hair follicle stem cell niches is required for the activation of

the Jagged-1-Notch signaling pathway, which in turn mediates hair follicle regeneration.³⁹ Tregs around the hair follicle are activated in the transition from telogen to anagen. These activated Tregs produce the Notch ligand Jagged-1 that stimulates the proliferation and differentiation of hair follicle stem cells and progression to the anagen phase.³⁴

Our findings of high Jagged-1 immunoexpression in large RS-L cells and that of Notch1 immunostaining in both epithelial and RS-L cells support the role of the Notch pathway in the proliferation of both cell types that contribute to tumor growth. Interestingly, Notch1 and Jagged-1 are also immunodetected in Reed-Sternberg cells of Hodgkin lymphoma in which they are known to block the expression of B-cell differentiation markers^{40,41} and are involved in Reed-Sternberg cell survival.⁴¹ Similarly, RS-L cells present in the cutaneous lymphadenoma do not express lymphocyte or epithelial markers. However, as a

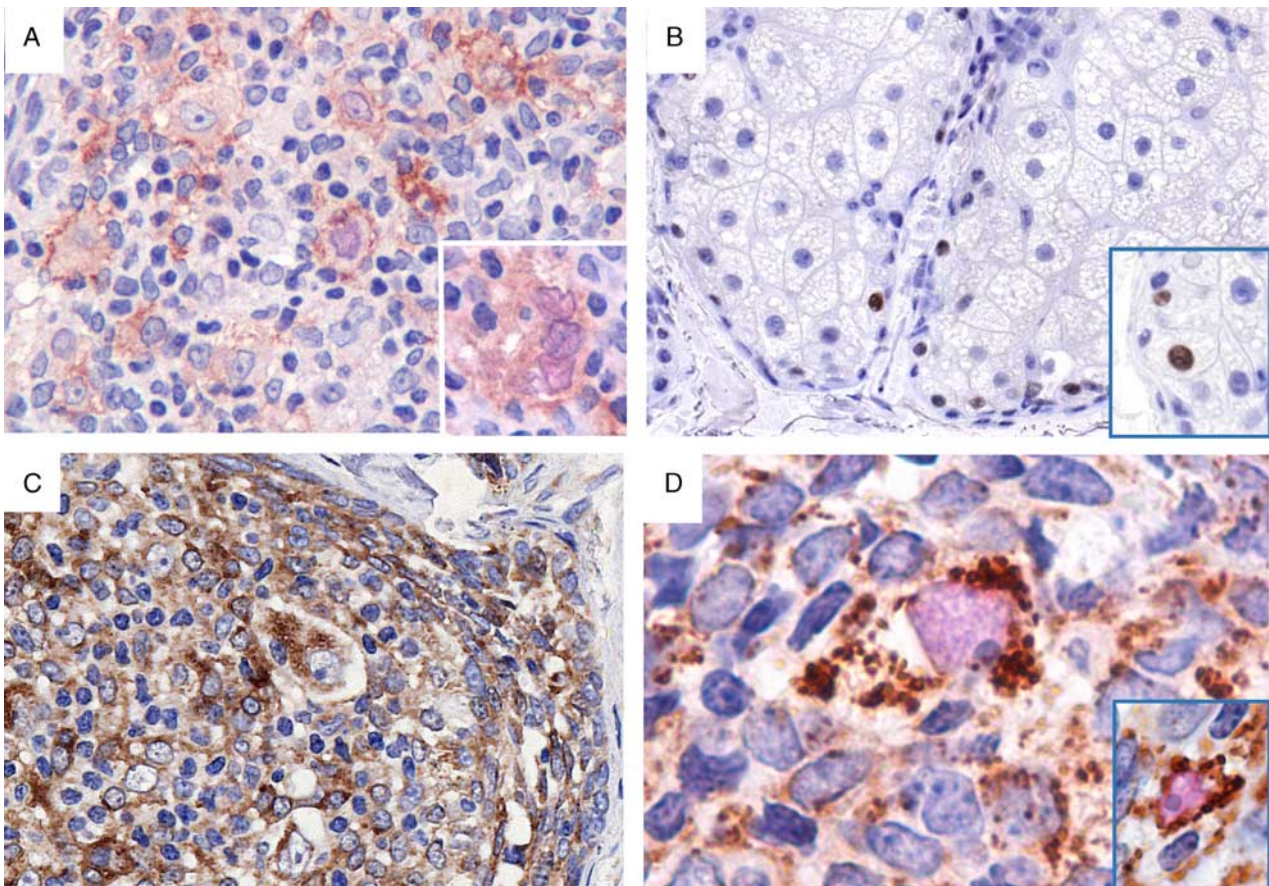


FIGURE 4. A, Nuclear NKX3.1 (magenta) is present in a subset of RS-L cells (center and inset), and membranous-cytoplasmic CD30 (brown) reactivity is also found in a few of them. B, NKX3.1 immunostaining in some peripheral sebocytes (inset) of adjacent normal sebaceous glands. C, Notch1 immunoreactivity in epithelial cells and in RS-L cells. D, Strong cytoplasmic Jagged-1 immunostaining (brown) in RS-L cells, some of which also exhibit nuclear NKX3.1 immunoreactivity (center and inset; magenta) (A, Double immunohistochemistry with NKX3.1-magenta chromogen and CD30-DAB chromogen; B and C, immunoperoxidase with DAB chromogen; D, double immunohistochemistry with NKX3.1-magenta chromogen and Jagged-1-DAB chromogen).

TABLE 4. Gene Mutations

Case #	EGFR	FGFR3	PIK3CA	ALK	MTOR	YAK1/3	MET	ERBB3	FGFR4
1									
2									
3									
4									
5									
6									
7									
8									
11									

Pathogenic mutations: green; nonpathogenic (benign or unknown clinical significance) mutations: brown.

subset of RS-L cells in the cutaneous lymphadenoma are positive for NKX3.1 and this androgen-regulated molecule is associated with a dedifferentiated or stem cell state and has been detected in hematopoietic stem cells and double-negative T lymphocytes,⁴² there is a possibility that RS-L cells, which are the main source of Notch1 ligand in cutaneous lymphadenoma, might be undifferentiated or stem hematopoietic cells, a hypothesis that has been previously proposed.^{43,44}

No genetic analysis of cutaneous lymphadenoma has been reported. Regarding our findings of EGFR, FGFR3, and PIK3CA somatic mutations in most cases of cutaneous lymphadenoma, it must be emphasized that although FGFR3 and PIK3CA mutations are common in a variety of benign skin lesions^{45,46} and even in sun-exposed normal skin,^{47,48} EGFR mutations have not been reported in either sun-exposed or non-sun-exposed normal skin⁴⁸ or in benign cutaneous adnexal tumors and, moreover, they are very unusual in cutaneous adnexal cancer.⁴⁹ In fact, in cutaneous melanoma, the skin cancer with the

TABLE 5. EGFR Mutations

Case #	EGFR Mutation	Coverage	Allele Ratio (%)	COSMIC
1	p.Asp761Asn	139	13	COSM21984
2	p-Ser768Asn	247	6	COSM12989
3	p.Cys797Tyr	455	4	COSM53104
4	p.Ala289Val	202	21	COSM21685
7	p.Asp761Asn	215	8	COSM21984

highest prevalence of EGFR mutations, only 6.5% of cases have EGFR mutations,⁵⁰ a rate which is much lower than we have found in cutaneous lymphadenoma (56%).

The EGFR hotspot missense mutations we found in cutaneous lymphadenoma are pathogenic.¹⁴ EGFR is involved in both AR and Notch signaling.¹⁴ Moreover, EGFR is required for the proliferation and appropriate coordination of molecular pathways involved in hair follicle development and adult hair follicle stem cell activation and growth,⁵¹ and there is evidence for a role of EGFR signaling in prevention of DNA damage in proliferating cells and susceptibility of epidermal cells to TP53-mediated apoptosis.⁵¹ Interestingly, although EGFR signaling is indispensable for the initiation of hair growth, continuous EGFR expression prevents entry into catagen phase.⁵² Therefore, we hypothesize that the EGFR gain-of-function somatic mutations may provoke a continuous hair follicle stem cell proliferation state and block entry into the catagen phase, which together with the Notch pathway stimulation, may be responsible for cutaneous lymphadenoma tumor growth. An alternative explanation is that cutaneous lymphadenoma arises from a nonbulge hair follicle stem cell keratinocyte progenitor that acquires genetic hits (eg, EGFR mutations) which then endow it with hair follicle bulge stem cell-like properties.

In summary, cutaneous lymphadenoma is a distinct lymphoepithelial tumor characterized by a proliferation of AR and Notch1-positive epithelial cells which express markers of hair follicle bulge stem cells, with numerous intralobular Tregs and Jagged-1-positive RS-L cells, commonly associated with EGFR somatic mutations. Furthermore, besides clear-cut differential histopathologic features, the presence of diffuse AR immunostaining easily allows its distinction from trichoblastoma.

ACKNOWLEDGMENTS

The authors acknowledge the enthusiastic support received in December 2019 from Daniel Santa Cruz when contacted and asked for his participation in this study. Although he could not further contribute or read the manuscript, this paper would be a posthumous homage to Daniel, who stated that cutaneous lymphadenoma was, in his own words, “one of his favorite sons.” The authors also acknowledge Laura Martínez, Esther Alvarez, and Maria José Gastaldo for their excellent assistance for the immunohistochemical and molecular biology techniques.

REFERENCES

1. Santa Cruz DJ, Barr RJ, Headington JT, et al. Cutaneous lymphadenoma. *Am J Surg Pathol*. 1991;15:101–110.

2. Masouyé I. Cutaneous lymphadenoma: report of 2 cases. *Dermatology*. 1992;185:62–65.
3. Botella R, Mackie RM. Cutaneous lymphadenoma: a case report and review of the literature. *Br J Dermatol*. 1993;128:339–341.
4. Okamura JM, Barr RJ. Cutaneous lymphoepithelial neoplasms. *Adv Dermatol*. 1997;12:277–294.
5. Pujol RM, Matias-Guiu X, Taberner R, et al. Benign lymphoepithelial tumor of the skin (“cutaneous lymphadenoma”). *Dermatol Online J*. 1999;5:5.
6. Wick MR, Santa Cruz DJ, Gru AA. Non-lymphoid lesions that may mimic cutaneous hematopoietic neoplasms histologically. *Semin Diagn Pathol*. 2017;34:99–107.
7. Fernandez-Flores A, Nicklaus-Wollenteit I, Sathishkumar D, et al. Congenital cutaneous lymphadenoma. *J Cutan Pathol*. 2017;44:954–957.
8. Diaz-Cascado C, Borghi S, Rey-Lopez A, et al. Cutaneous lymphadenoma. A peculiar variant of nodular trichoblastoma. *Am J Dermatopathol*. 1996;18:186–191.
9. Soyer HP, Kutzner H, Jacobson M, et al. Cutaneous lymphadenoma is adamantinoid trichoblastoma. *Dermatol Pract Concept*. 1996;2:32–38.
10. Betti R, Alessi E. Nodular trichoblastoma with adamantinoid features. *Am J Dermatopathol*. 1996;18:192–195.
11. McNiff JM, Eisen RN, Glusac EJ. Immunohistochemical comparison of cutaneous lymphadenoma, trichoblastoma, and basal cell carcinoma: support for classification of lymphadenoma as a variant of trichoblastoma. *J Cutan Pathol*. 1999;26:119–124.
12. Keesecker S, Saab J, Magro CM, et al. Cutaneous lymphadenoma: a trichoblastoma with regressive inflammatory changes. *Facial Plast Surg*. 2017;33:109–111.
13. Meyer KC, Klatte JE, Dinh HV, et al. Evidence that the bulge region is a site of relative immune in human hair follicles. *Br J Dermatol*. 2008;159:1077–1085.
14. Catalogue of somatic Mutations in Cancer (COSMIC v92). 2020. Available at: <https://cancer.sanger.ac.uk/cosmic/mutation>. Accessed August 27, 2020.
15. Calonje E, Brenn T, Lazar A, Billings S, eds. *McKee's Pathology of the Skin With Clinical Correlations*, 5th ed. Amsterdam, NL: Elsevier; 1583–1584.
16. Kazakov DV, Banik M, Kacerovska D, et al. A cutaneous adnexal neoplasm with features of adamantinoid trichoblastoma (lymphadenoma) in the benign component and lymphoepithelial-like carcinoma in the malignant component: a possible case of malignant transformation of a rare trichoblastoma variant. *Am J Dermatopathol*. 2011;33:729–732.
17. Evangelista MT, North JP. Comparative analysis of cytokeratin 15, TDAG51, cytokeratin 20 and androgen receptor in sclerosing adnexal neoplasms and variants of basal cell carcinoma. *J Cutan Pathol*. 2015;42:824–831.
18. Katona TM, Perkins SM, Billings SD. Does the panel of cytokeratin 20 and androgen receptor antibodies differentiate desmoplastic trichoepithelioma from morpheiform/infiltrative basal cell carcinoma? *J Cutan Pathol*. 2008;35:174–179.
19. Jih DM, Lyle S, Elenitsas R, et al. Cytokeratin 15 expression in trichoepitheliomas and a subset of basal cell carcinomas suggests they originate from hair follicle stem cells. *J Cutan Pathol*. 1999;26:113–118.
20. Izikson L, Bhan A, Zembowicz A. Androgen receptor expression helps to differentiate basal cell carcinoma from benign trichoblastic tumors. *Am J Dermatopathol*. 2005;27:91.
21. Sellheyer K, Nelson P, Kutzner H, et al. The immunohistochemical differential diagnosis of microcystic adnexal carcinoma, desmoplastic trichoepithelioma and morpheiform basal cell carcinoma using BerEP4 and stem cell markers. *J Cutan Pathol*. 2013;40:363–370.
22. Wang Q, Ghimire D, Wang J, et al. Desmoplastic trichoepithelioma: a clinicopathological study of three cases and a review of the literature. *Oncol Lett*. 2015;10:2468–2476.
23. Stanoszek LM, Wang GY, Harms PW. Histologic mimics of basal cell carcinoma. *Arch Pathol Lab Med*. 2017;141:1490–1502.
24. Katona TM, Ravis SM, Perkins SM, et al. Expression of androgen receptor by fibroepithelioma of Pinkus: evidence supporting classification as a basal cell carcinoma variant? *Am J Dermatopathol*. 2007;29:7–12.

25. Sellheyer K, Nelson P, Kutzner H. Fibroepithelioma of Pinkus is a true basal cell carcinoma developing in association with a newly identified tumour-specific type of epidermal hyperplasia. *Br J Dermatol*. 2012;166:88–97.
26. Inui S, Itami S. Androgen actions on the human hair follicle: perspectives. *Exp Dermatol*. 2013;22:168–171.
27. Thornton MJ, Taylor AH, Mulligan K, et al. The distribution of estrogen receptor beta is distinct to that of estrogen receptor alpha and the androgen receptor in human skin and the pilosebaceous unit. *J Invest Dermatol Symp Proc*. 2003;8:100–103.
28. Bayer-Garner IB, Givens V, Smoller B. Immunohistochemical staining for androgen receptors: a sensitive marker of sebaceous differentiation. *Am J Dermatopathol*. 1999;21:426–431.
29. Kretschmar K, Cottle DL, Schweiger PJ, et al. The androgen receptor antagonizes Wnt/beta-catenin signaling in epidermal stem cells. *J Invest Dermatol*. 2015;135:2753–2763.
30. Asada Y, Sonoda T, Ojio M, et al. 5 alpha-reductase type 2 is constitutively expressed in the dermal papilla and connective tissue sheath of the hair follicle in vivo but not during culture in vitro. *J Clin Endocrinol Metab*. 2001;86:2875–2880.
31. Gurel B, Ali TZ, Montgomery EA, et al. NKX3.1 as a marker of prostatic origin in metastatic tumors. *Am J Surg Pathol*. 2010;34:1097–1105.
32. Gelmann EP, Bowen C, Bubendorf L. Expression of NKX3.1 in normal and malignant tissue. *Prostate*. 2003;55:111–117.
33. Fujimura T, Itoigawa A, Haga T, et al. Cutaneous lymphadenoma: a case report and immunohistochemical study. *Case Rep Dermatol*. 2012;4:50–55.
34. Ali N, Zirak B, Rodriguez RS, et al. Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell*. 2017;169:1119.e11–1129.e11.
35. Castellana D, Paus R, Perez-Moreno M. Macrophages contribute to the cyclic activation of adult hair follicle stem cells. *PLoS Biol*. 2014;12:e1002002.
36. Jahoda CA, Christiano AM. Niche crosstalk: intercellular signals at the hair follicle. *Cell*. 2011;146:678–681.
37. Senechal B, Elain G, Jeziorski E, et al. Expansion of regulatory T cells in patients with Langerhans cell histiocytosis. *PLoS Med*. 2007;4:e253.
38. Plikus MV, Gay DL, Treffeisen E, et al. Epithelial stem cells and implications for wound repair. *Semin Cell Dev Biol*. 2012;23:946–953.
39. Maryanovich M, Frenette PS. T-regulating hair follicle stem cells. *Immunity*. 2017;46:979–981.
40. Jundt F, Anagnostopoulos I, Förster R, et al. Activated Notch1 signaling promotes tumor cell proliferation and survival in Hodgkin and anaplastic large cell lymphoma. *Blood*. 2002;99:3398–3403.
41. Schwarzer R, Dörken B, Jundt F. Notch is an essential upstream regulator of NF-κB and is relevant for survival of Hodgkin and Reed-Sternberg cells. *Leukemia*. 2012;26:806–813.
42. Nagel S, MacLeod RAF, Meyer C, et al. NKX homeobox gene activities in B-cell development and lymphomas. *PLoS One*. 2018;13:e0205537.
43. Rodriguez-Díaz E, Román C, Yuste M, et al. Cutaneous lymphadenoma: an adnexal neoplasm with intralobular activated lymphoid cells. *Am J Dermatopathol*. 1998;20:74–78.
44. Alexis J. Immunohistochemical profile of an adamantinoid trichoblastoma (cutaneous lymphadenoma). *J Cutan Pathol*. 1999;26:477.
45. Hafner C, López-Knowles E, Luis NM, et al. Oncogenic PIK3CA mutations occur in epidermal nevi and seborrheic keratoses with a characteristic mutation pattern. *Proc Natl Acad Sci USA*. 2007;104:13450–13454.
46. Heidenreich B, Denisova E, Rachakonda S, et al. Genetic alterations in seborrheic keratoses. *Oncotarget*. 2017;8:36639–36649.
47. Martincorena I, Roshan A, Gerstung M, et al. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science*. 2015;348:880–886.
48. Yizhak K, Aguet F, Kim J, et al. RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. *Science*. 2019;364:eaaw0726.
49. Cavalieri S, Busico A, Capone I, et al. Identification of potentially druggable molecular alterations in skin adnexal malignancies. *J Dermatol*. 2019;46:507–514.
50. Liu H, Zhang B, Sun Z. Spectrum of EGFR aberrations and potential clinical implications: insights from integrative pan-cancer analysis. *Cancer Commun*. 2020;40:43–59.
51. Amberg N, Sotiropoulou PA, Heller G, et al. EGFR controls hair shaft differentiation in a p53-independent manner. *iScience*. 2019;15:243–256.
52. Mak KK, Chan SY. Epidermal growth factor as a biologic switch in hair growth cycle. *J Biol Chem*. 2003;278:26120–26126.