Patterns of LC3A Autophagy Protein Expression in Keratoacanthomas

Efthimios Sivridis¹ · Ioannis M. Koukourakis¹ · Stella Arelaki¹ · Kostantina Balaska¹ · Antonios Karpouzis² · Alexandra Giatromanolaki¹

Received: 21 October 2018 / Accepted: 18 March 2019 / Published online: 11 April 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

To investigate the expression patterns of autophagy marker light chain protein 3 (LC3A) in keratoacanthoma (KA). KAs are generally regarded as benign but malignant behavior, including rare metastases, may occur. 85 KAs were assessed for the LC3A autophagic protein by immunohistochemistry. Diffuse cytoplasmic staining and a “stone-like structure” (SLS) characterized positive expression. Thirty-four out of 85 KAs (40%) had diffuse cytoplasmic LC3A immunostaining (percentage of positive cells ranging from 5 to 60%). In contrast, only 4 of the 85 KAs (4.7%) expressed SLSs. Only one SLS was detected per histologic section of each tumor. The p53 oncoprotein was encountered in all cases with expression ranging from 1 to 90% of cells (median 30%). The Ki-67 index was expressed in 63 cases (74% of cases; range 1–50% of cells; median value 5%). Neither of these two parameters nor diffuse cytoplasmic LC3A staining was significantly correlated with SLS expression or lack thereof. Expression of SLSs, a hallmark of malignancy, was found in 4.7% of KAs. Further study is necessary to determine whether this fraction represents the exceptional cases that harbor latent malignant potential.

Keywords Keratoacanthoma · Autophagy · LC3A · Stone-like structures · P53 · Ki67

Introduction

Keratoacanthoma (KA) is a common cutaneous lesion of unclear etiology [1, 2]. These lesions affect mainly, if not exclusively, elderly populations. KAs occur in sun-exposed areas of the skin as solitary, dome-shaped tumors, 1–2 cm in diameter, with a large, keratin-filled central crater. They show a remarkable morphologic similarity with well-differentiated squamous cell carcinoma (SCC) which may suggest malignancy. Their characteristic rapid growth further supports this impression, but these lesions characteristically spontaneously involute with rare exceptions [3]. There are few reports of recurrence, malignant transformation, and metastasis [4, 5].

The unusual behavior of these lesions has raised two critical questions: (a) What is the true nature of KAs? (b) If malignant, is there a method to detect which tumors are likely to metastasize? Despite extensive research on the subject, opinions as to the nature of this disease vary. Some authorities believe KA is a distinct, self-resolving, benign squamous cell lesion [1, 6–12]. Other authors categorize it as a variant of SCC with a definite, although rare, potential for metastasis [4, 5, 13]. Those that subscribe to the latter view are skeptical of self-regressing KAs due to common early surgical intervention. They argue the so-called metastasizing KAs are, in fact, SCCs misdiagnosed as KAs, and some further propose the diagnosis “well differentiated squamous cell carcinoma, keratoacanthoma type” [3, 6, 11, 14, 15].

Light chain protein 3 (LC3A) is an essential component of the autophagosomes. We recently demonstrated this protein to be a tumor-specific marker for epithelial malignancies and their immediate precursors when expressed in the form of “stone-like structures” (SLSs). SLSs are large, round, and densely-stain with antibodies for LCA3 by immunohistochemistry. These structures are typically enclosed within a cytoplasmic vacuole (autophagosome) and may be identified in one or more cells after an extensive search of the

✉ Efthimios Sivridis
esivrid@med.duth.gr

¹ Departments of Pathology, Democritus University of Thrace Medical School and University General Hospital of Alexandroupolis, 68100 Alexandroupolis, Greece

² Departments of Dermatology, Democritus University of Thrace Medical School and University General Hospital of Alexandroupolis, 68100 Alexandroupolis, Greece

entire histological section. No more than one SLS is needed to characterize an individual case as “stone-like” LC3A positive, but increased numbers are associated with high tumor grade and an unfavorable prognosis. Other patterns of autophagic LC3A expression, namely diffuse cytoplasmic and cytoplasmic perinuclear, are non-specific and may be observed in normal, benign, and malignant tissues [16].

These findings are supported by similar observations of SLSs in malignancies of the breast, prostate, large intestine, cutaneous SCC, lung, urinary bladder, and brain [16–24]. One group verified the presence of SLSs with different methods, including electron microscopy, tumor spheroids, xenografts, and tumor cell lines [28].

Autophagy is an intracellular degradation mechanism whereby cytoplasmic contents are delivered to lysosomes (autophagosomes), and the resulting macromolecular constituents are recycled [29, 30]. This process operates constitutively at a basal level and may be identified through immunohistochemistry as a diffuse cytoplasmic staining pattern with anti-LC3A antibodies [31, 32]. Autophagy is upregulated under conditions of stress, including limitation or deprivation of nutrients, as damaged proteins and organelles accumulate. SLSs are encountered in circumstances of high cellular turnover with malignant and pre-malignant epithelial tissues.

Because of the strong links between upregulated autophagy and neoplasia, we were interested in the immunohistochemical LC3A patterns expressed by KAs [16–27]. We also examined p53 oncoprotein expression and Ki-67 as a proliferation marker [33–35]. Playing an essential role in cell cycle control and apoptosis, p53 is the most frequently altered gene in human cancers. Defects in this protein allow abnormal cells to proliferate and are associated with malignant neoplasia. The expression of Ki-67 is also intimately associated with tumor cell proliferation and growth.

Materials and Methods

Our study comprised a series of 85 excisional biopsy specimens of KAs previously diagnosed by routine hematoxylin and eosin (H&E) stains. Blocks were retrieved from the files of the Department of Pathology, Democritus University of Thrace Medical School, Alexandroupolis, Greece. The tissues had been fixed in 10% formal saline and processed conventionally to paraffin wax. They were evaluated for the expression of the autophagic protein LC3A as well as antigens Ki-67(MIB1) and p53 using immunohistochemistry. The ethics committees of the institution approved the study.

Immunohistochemical Techniques for LC3A, Ki-67 and p53

LC3A reactivity was detected by a standard immunohistochemical technique using the purified rabbit polyclonal cleaved antibody MAP1LC3A (AP1805a) (Abgent, San Diego, CA). Three micron thick tissue sections were cut and placed on positively charged slides then deparaffinized and rehydrated through graded alcohols to water. For heat-induced epitope retrieval, the sections were placed in citrate buffer (DAKO EnVision FLEX Target Retrieval Solution, 1:10 dilution, pH 6) and heated at 97 °C (3×5 min). Subsequently, slides were left to cool at room temperature for 20 min. Immunohistochemistry of the LC3A antibody was performed using the Ultra Vision Quanto Detection System. Nonspecific binding was blocked by preincubation with UltraVision Protein Block for 10 min at room temperature. The slides were then incubated overnight at 4 °C with MAP1LC3A (AP1805a) primary antibody diluted 1:100 (Abgent). After overnight incubation, tissue sections were buffered washed (2×6 min) in phosphate-buffered saline (PBS). UltraVision Hydrogen Peroxide Block was then applied for 10 min to neutralize endogenous peroxidase activity. After washes in PBS (2×6 min), the sections were incubated with Primary Antibody Amplifier Quanto for 10 min at room temperature to enhance the penetration of the subsequently applied polymer. Slides were subsequently washed in PBS (2×6 min) and incubated with HRP Polymer Quanto for 10 min at room temperature. This step recognizes mouse and rabbit immunoglobulins and detects any tissue-bound primary antibody. After thorough washing in PBS (3×6 min), the color reaction was developed in 3,3'-diaminobenzidine tetrahydrochloride (DAB Quanto Chromogen) for 6 min. Finally, a buffer wash was performed (2×6 min) and the sections were counterstained with hematoxylin OS (Vector H-3404), dehydrated, and mounted.

Evaluation of p53 and of the proliferative activity (Ki-67 index) was performed using the DAKO EnVision FLEX detection system. The mouse monoclonal antibody p53 (DAKO, clone DO-7) was applied at a dilution of 1:30 for 60 min at room temperature. The mouse monoclonal antibody Ki-67 (DAKO, clone MIB-1) was applied at a dilution of 1:100 for 60 min at room temperature.

Controls

In all cases, appropriate positive and negative controls were included in each staining run. Specifically, normal rabbit immunoglobulin-G was substituted for the primary antibody as a negative control. Tissues from cutaneous SCCs were used as positive controls.

Evaluation of Immunohistochemical Stainings

Staining for the LC3A protein revealed two distinct patterns of cytoplasmic expression in KAs: diffuse cytoplasmic staining and SLSs within cytoplasmic vacuoles. In all cases, evaluation of the diffuse staining pattern was performed after examining the entire sections at $\times 200$ magnification. Reactivity was expressed as a proportion of positive cells.

Immunohistochemical expression of Ki-67 and p53 proteins was invariably nuclear and comparable to positive controls. The percentage of tumor cells with Ki-67 and p53 expression was assessed in all available fields at $\times 200$ magnification, and the mean value was used to score each case. The LC3A, Ki-67, and p53 immunoreactivities were evaluated independently by three observers (ES, SA, and IMK), and any discrepancies were resolved on the conference microscope.

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism 5.0 and the InStat 3.1 package (GraphPad Software, Inc.). The Fisher exact test or the unpaired 2-tailed t-test was used for testing relationships between categorical variables as appropriate. Linear regression analysis was used to assess correlation with continuous variables. A *P* value of < 0.05 was used for significance.

Results

The patients' age varied from 50 to 93 years (mean 73.06 years). There were 54 men (mean age 73.4) and 31 women (mean age 72.46).

LC3A Expression Patterns in KAs

Two distinct patterns of autophagic activity were identified in KAs by LC3A expression: diffuse cytoplasmic staining and the stone-like pattern. The first pattern was seen in 40% of KAs (34/85) as a granular brown stain throughout the entire cytoplasm of the tumor cells (Fig. 1a). The mean percentage of neoplastic cells with a cytoplasmic expression was 7.8% (range 0–70%; median 0%).

In contrast, SLSs were detected in only 4 of the 85 cases (4.7%). The reaction was recognized as a dense, round, amorphous material enclosed within a cytoplasmic vacuole (autophagosome) (Fig. 1b, c). The size of SLSs was on average 5 μ m diameter. In all four cases, one SLS was identified

in the entire histological section. Perinuclear staining was not noted in our KA series.

Ki-67 (MIB1) Index and the p53 Oncoprotein

As expected, the staining pattern of Ki-67 (MIB1) and p53 protein was invariably nuclear (Fig. 1d, e). The p53 oncoprotein was encountered in all cases, but the proportion of positive cases ranged from 1 to 90% (median value 30%). Ki-67 was expressed in 63/85 cases (74%), with the proportion of reactive cells ranging between 1–50% (median 5%). Neither of these two parameters were significantly associated with SLS expressing or non-expressing KAs. Moreover, similar results were obtained with the LC3A diffuse cytoplasmic staining. The distribution of MIB1, p53, and cytoplasmic LC3A according to the presence of SLS and the linear regression analysis between parameters is shown in Fig. 2.

Discussion

In this study, cutaneous KAs were investigated in relation to the light chain 3 (LC3A) protein, an essential component of autophagosomes. The autophagy process is complementary to oxidative phosphorylation, angiogenesis, and anaerobic glycolysis. It is recorded at a basal energy rate, expressed as the diffuse LC3A cytoplasmic staining pattern, and at a high rate in the form of SLSs. At basal levels (diffuse cytoplasmic expression), autophagic degradation maintains cellular homeostasis in most cell types, normal, benign and malignant, through the clearance of damaged proteins and organelles. Elevated autophagy is seen in response to stressors including oncogenic or metabolic insults. This process may contribute to tumor initiation and growth by promoting cell survival in malignant and premalignant lesions. In this context, the SLS pattern has been repeatedly associated with aggressive tumors and an unfavorable prognosis [16, 19, 22, 24–28]. Whether the SLS pattern is representative of high energy compounds derived from active autophagic functions or merely the waste products of such compounds is a moot point as increased numbers of SLSs reflect tumor aggression in malignancy or progression in premalignant lesions [36]. The presence of SLSs in KAs may be telling the true nature of this disease.

We examined 85 KAs by for LC3A expression by immunohistochemistry. The tumors studied were largely of low turnover as inferred by a diffuse cytoplasmic LC3A staining pattern. SLSs of high turnover tumors were identified in a low percentage of KAs (4/85 or 4.7%), with only one SLS in each positive histological section. Interestingly, low SLS counts, no more than one or two per histological section, are documented in pre-invasive disease. These lesions are characterized by cellular atypia without true tissue invasion. The

Fig. 1 Reactivity patterns of different markers in keratoacanthoma by immunohistochemistry. **a** A squamous cell skin carcinoma used as control to highlight cytoplasmic/perinuclear LC3A staining (thin arrows) and a typical LC3A positive SLS (thick arrow); **b** LC3A

reactivity showing a diffuse, granular, brown stain of the entire cytoplasm of keratoacanthoma cells; **c, d** LC3A positive SLSs (thick arrows); **e** typical Ki-67 immunostaining of keratoacanthoma cell nuclei; **f** Nuclear accumulation of p53 protein in neoplastic cells

presence of SLSs in pre-invasive lesions may be indicative of progression to low-grade malignancy [24].

These findings stem from our own immunohistochemical studies as well as those of others including different tumor types such as adenocarcinomas of the colon, endometrium, and prostate, non-small cell carcinomas (NSCC) of the lung, urothelial carcinomas, astrocytomas, and squamous cell carcinomas of the larynx and skin. In the study of 75 cutaneous SCCs, malignancies with a close resemblance to KA, all expressed SLSs [19]. Counts ranged between 1 and 20 SLSs per histological section (median 4) [19]. Interestingly, the highest SLS counts (4–20 per histological section; median 9) were encountered in tumors greater than 6 mm thick [37]. Tumors greater than 6 mm in thickness are associated with a high risk of metastasis and local recurrence [37].

Additional studies from our group and others confirmed increasing SLS counts per section when neoplastic lesions progress from pre-invasive to invasive patterns of growth. For example, endometrial hyperplasia with cellular atypia, a

pre-invasive lesion, showed small numbers of SLSs per section [23]. Low SLS numbers per section were also recorded in non-transformed, non-invasive areas of dysplastic colonic tubular adenomas [24]. The SLS numbers progressively increased between areas of equivocal invasion to unequivocal, invasive foci in malignant lesions [24].

KAs are a complex disease of contradictory nature regarding malignant potential. Given the low propensity for progression and metastasis, it is not surprising that only a few SLSs, both in incidence and number per section, were detected in KAs. The question of whether the few KAs expressing SLSs have increased malignant potential demands evaluation in a series of patients with documented invasive progression. Such lesions, which commonly pursue an indolent clinical course but retain a small potential for unexpected metastases, are known to occur in tumor pathology. They are characterized by the World Health Organization (WHO) as “tumors of uncertain malignant potential, rarely metastasizing.” Tumors of this type include solitary

Fig. 2 Correlation between parameters (percentage of keratoacanthoma cells with positive expression assessed in all available optical fields): **a** distribution of MIB1, p53, and cytoplasmic LC3A according to the presence of SLS (bars show standard deviation); **b**, **c**, **d** linear regression analysis between the % of keratoacanthoma cells with cytoplasmic LC3A vs. MIB1 (**b**), cytoplasmic LC3A vs. p53 (**c**), and p53 vs. MIB1 (**d**)

fibrous tumors, granular cell tumors, angiomyolipoma/PEComa, and inflammatory myofibroblastic tumors, among others [38–45]. We consider KA, with scanty SLSs and the clinicopathologic features outlined above, as a member of this diagnostic category. With a proven association between SLSs and malignant progression, prediction of the behavior of KAs may be made at diagnosis. Other markers, such as p53 or Ki-67 index are frequently expressed in KAs and unlikely to identify ‘high risk’ lesions. Although cellular atypia and mitoses are relatively common in KAs, it is unclear whether these features relate to malignant progression [46–48].

The current findings are in agreement with the concept that KAs are, by and large, benign lesions. A small proportion of cases harbor malignant potential which, if stimulated by appropriate endogenous and exogenous factors, may progress and result in unexpected metastases. Similar lesions are classified by the WHO as tumors of intermediate biological behavior, rarely metastasizing. It is our opinion that KA should be included in this group. As no specific feature can predict malignant progression at the time of excision, it may prove useful to evaluate KAs for the presence of SLSs. Further studies on patients with

KA that clinically progressed are demanded to validate SLS as a biomarker of malignancy for KAs.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Savage JA, Maize JC, Sr. Keratoacanthoma clinical behavior: a systematic review. *Am J Dermatopathol.* 2014;36:422–9.
2. Oellers P, Karp CL, Shah RR, et al. Conjunctival keratoacanthoma. *Br J Ophthalmol.* 2014;98:275–6.
3. Ko CJ. Keratoacanthoma: facts and controversies. *Clin Dermatol.* 2010;28:254–61.
4. Piscioi F, Boi S, Zumiani G, et al. A gigantic, metastasizing keratoacanthoma. Report of a case and discussion on classification. *Am J Dermatopathol.* 1984;6:123–9.
5. Hodak E, Jones RE, Ackerman AB. Solitary keratoacanthoma is a squamous-cell carcinoma: three examples with metastases. *Am J Dermatopathol.* 1993;15:332–42.

6. Mandrell JC, Santa Cruz D. Keratoacanthoma: hyperplasia, benign neoplasm, or a type of squamous cell carcinoma? *Semin Diagn Pathol.* 2009;26:150–63.
7. Weedon D. Weedon's skin pathology. Keratoacanthoma centrifugum marginatum. Edinburgh: Churchill Livingstone Elsevier; 2010. p. 702–8.
8. Godbolt AM, Sullivan JJ, Weedon D. Keratoacanthoma with perineural invasion: a report of 40 cases. *Australas J Dermatol.* 2001;42:168–71.
9. Weedon D, Malo J, Brooks D, et al. Keratoacanthoma: is it really a variant of squamous cell carcinoma? *ANZ J Surg.* 2010;80:129–30.
10. Ra SH, Su A, Li X, et al. Keratoacanthoma and squamous cell carcinoma are distinct from a molecular perspective. *Mod Pathol.* 2015;28:799–806.
11. Gleich T, Chiticariu E, Huber M, et al. Keratoacanthoma: a distinct entity? *Exp Dermatol.* 2016;25:85–91.
12. Ogita A, Ansai SI, Misago N, et al. Histopathological diagnosis of epithelial crateriform tumors: Keratoacanthoma and other epithelial crateriform tumors. *J Dermatol.* 2016;43:1321–31.
13. Manstein CH, Fraumeni CJ, Besden JE. Keratoacanthoma: is it a real entity? *Ann Plast Surg.* 1998;40:469–72.
14. Sarma D. Keratoacanthoma should be reported as “well differentiated squamous cell carcinoma, Keratoacanthoma Type”: a dermatopathologist's view. *Internet J Dermatol.* 2006;5:1.
15. Goldenberg G, Patel S, Patel MJ, et al. Eruptive squamous cell carcinomas, keratoacanthoma type, arising in a multicolor tattoo. *J Cutan Pathol.* 2008;35:62–4.
16. Sivridis E, Koukourakis MI, Zois CE, Ledaki I, Ferguson DJ, Harris AL, Gatter KC, Giatromanolaki A. LC3A-positive light microscopy detected patterns of autophagy and prognosis in operable breast carcinomas. *Am J Pathol.* 2010;176:2477–89.
17. Giatromanolaki A, Sivridis E, Mendrinis S, et al. Autophagy proteins in prostate cancer: relation with anaerobic metabolism and Gleason score. *Urol Oncol.* 2014;32:39.e11–8.
18. Giatromanolaki A, Koukourakis MI, Harris AL, et al. Prognostic relevance of light chain 3 (LC3A) autophagy patterns in colorectal adenocarcinomas. *J Clin Pathol.* 2010;63:867–72.
19. Sivridis E, Giatromanolaki A, Karpithou G, et al. LC3A-positive “stone-like” structures in cutaneous squamous cell carcinomas. *Am J Dermatopathol.* 2011;33:285–90.
20. Karpithou G, Sivridis E, Koukourakis MI, et al. Light-chain 3A autophagic activity and prognostic significance in non-small cell lung carcinomas. *Chest.* 2011;140:127–34.
21. Sivridis E, Koukourakis MI, Mendrinis SE, et al. Patterns of autophagy in urothelial cell carcinomas—the significance of “stone-like” structures (SLS) in transurethral resection biopsies. *Urol Oncol.* 2013;31:1254–60.
22. Giatromanolaki A, Sivridis E, Mitrakas A, et al. Autophagy and lysosomal related protein expression patterns in human glioblastoma. *Cancer Biol Ther.* 2014;15:1468–78.
23. Sivridis E, Giatromanolaki A, Liberis V. Autophagy in endometrial carcinomas and prognostic relevance of ‘stone-like’ structures (SLS) What is destined for the atypical endometrial hyperplasia? *Autophagy.* 2011;7:74–82.
24. Giatromanolaki A, Koukourakis M, Koutsopoulos, et al. Autophagy and hypoxia in colonic adenomas related to aggressive features. *Colorectal Dis.* 2013;15:e223–30.
25. Liao W, Sun L, Wang C, Huang H, Liu J, Liao W, Shi M. LC3A-positive “stone-like” structures predict an adverse prognosis of gastric cancer. *Anat Rec (Hoboken).* 2014;297:653–62.
26. Xi SY, Lu JB, Chen JW, et al. The “stone-like” pattern of LC3A expression and its clinicopathologic significance in hepatocellular carcinoma. *Biochem Biophys Res Commun.* 2013;431:760–6.
27. Spowart JE, Townsend KN, Huwait H, et al. The autophagy protein LC3A correlates with hypoxia and is a prognostic marker of patient survival in clear cell ovarian cancer. *J Pathol.* 2012;228:437–47.
28. Sivridis E, Giatromanolaki A, Zois C, et al. The “stone-like” pattern of autophagy in human epithelial tumors and tumor-like lesions. An approach to the clinical outcome. *Autophagy.* 2010;6:122–5.
29. Feng Y, He D, Yao Z, Klionsky DJ. The machinery of macroautophagy. *Cell Res.* 2014;24:24–41.
30. Mizushima N. Autophagy: process and function. *Genes Dev.* 2007;21:2861–73.
31. Klionsky DJ, Baehrecke EH, Brumell JH, et al. A comprehensive glossary of autophagy-related molecules and processes (2nd ed.). *Autophagy* 2011;7:1273–94.
32. Musiwaro P, Smith M, Manifava M, et al. Characteristics and requirements of basal autophagy in HEK 293 cells. *Autophagy.* 2013;9:1407–17.
33. Li LT, Jiang G, Chen Q, et al. Ki67 is a promising molecular target in the diagnosis of cancer (review). *Mol Med Rep.* 2015;11:1566–72.
34. Joshi S, Schjølberg AR, Ekstrøm PO, et al. Tp53/p53 status in keratoacanthomas. *J Cutan Pathol.* 2016;43:571–8.
35. Watanabe IC, Magalhães RF, de Moraes AM, et al. Keratoacanthoma and keratoacanthoma-like squamous cell carcinoma: similar morphology but different pathogenesis. *Medicine (Baltimore).* 2015;94:e934.
36. Martinet W, Roth L, De Meyer GRY, Standard immunohistochemical assays to assess autophagy in mammalian tissue. *Cells.* 2017;6(3):17.
37. Brantsch KD, Meisner C, Schönfisch B, et al. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. *Lancet Oncol.* 2008;9:713–20.
38. Amorim E. Solitary fibrous tumor of the pleura: 3 case reports. *Rev Assoc Med Bras.* 2015;61:207–8.
39. Thway K, Ng W, Noujaim J, et al. The current status of solitary fibrous tumor: diagnostic features, variants, and genetics. *Int J Surg Pathol.* 2016;24:281–92.
40. Strickland KC, Nucci MR, Esselen KM, et al. Solitary fibrous tumor of the uterus presenting with lung metastases: a case report. *Int J Gynecol Pathol.* 2016;35:25–9.
41. Akahane K, Kato K, Ogiso S, Sakaguchi K, et al. Malignant granular cell tumor of the breast: case report and literature review. *Breast Cancer.* 2015;22:317–23.
42. Martignoni G, Pea M, Zampini C, et al. PEComas of the kidney and of the genitourinary tract. *Semin Diagn Pathol.* 2015;32:140–59.
43. Doyle LA, Hornick JL, Fletcher CD. PEComa of the gastrointestinal tract: clinicopathologic study of 35 cases with evaluation of prognostic parameters. *Am J Surg Pathol.* 2013;37:1769–82.
44. Do BA, Varshney R, Zawawi F, et al. Inflammatory myofibroblastic tumor of the larynx—a case report. *J Voice.* 2014;28:258–61.
45. Lai LM, McCarville MB, Kirby P, et al. Shedding light on inflammatory pseudotumor in children: spotlight on inflammatory myofibroblastic tumor. *Pediatr Radiol.* 2015;45:1738–52.
46. Strieth S, Hartschuh W, Pilz L, et al. Carcinoma-like vascular density in atypical keratoacanthoma suggests malignant progression. *Br J Cancer.* 2002;87:1301–7.
47. Chow TK, Chacko E, Cleary C, et al. Keratoacanthoma of the lower eyelid. *Eye (Lond).* 2005;19:689–90.
48. Clausen OP, Aass HC, Beigi M, et al. Are keratoacanthomas variants of squamous cell carcinomas? A comparison of chromosomal aberrations by comparative genomic hybridization. *J Invest Dermatol.* 2006;126:2308–15.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.