

Clinical and Immunohistochemical Features of Sebaceous Carcinoma: Focusing on the p53 Tumor Suppressor

Gobind Singh^{1*}, Brett Weinstock¹, Robert G. Phelps^{2,3,#} and Albert Y Wu^{1#}

¹Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, USA

²Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, USA

³Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, USA

#Authors contributed equally to manuscript

*Corresponding author: Gobind Singh, MD, PhD, Department of Ophthalmology, Harkness Eye Institute, Columbia University, 635 W 165th Street, New York, NY 10032, USA, E-mail: gs2924@cumc.columbia.edu

Received date: November 16, 2016; Accepted date: February 23, 2017; Published date: February 28, 2017

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Abstract

Purpose: Considerable focus has recently revealed the importance of p53 in sebaceous carcinoma (SC). This study investigates the utility of p53 as an immunohistochemistry (IHC) marker for differentiating benign and malignant sebaceous tissues and reports the clinical characteristics of patients diagnosed with SC at Mount Sinai Hospital (MSH).

Methods: A retrospective chart analysis of 102 patients with SC was performed in addition to a histopathological examination of benign and malignant sebaceous tissues. Immunohistochemistry was performed in 30 cases of SC, 4 cases of sebaceous hyperplasia, 4 cases of sebaceous epitheliomas, and 3 cases of sebaceous adenoma. A separate outpatient cohort was used to compare the clinical and histologic features of eyelid lesions. This included 31 available cases with histologic descriptions of SC. The clinical features of 20 SC cases, 25 BCC cases, and 21 chalazia cases were available and compared from this outpatient cohort. Data collected included age, gender, lesion anatomical location, histopathologic descriptions, and percentage of p53 positive sebocytes.

Results: Of the 102 SC cases at MSH, the mean age was 69 years, males composed 58%, and 59% were periorbital or orbital. Positive p53 staining without atypia was reproducibly observed at cell membranes in all cases of sebaceous hyperplasia. Using a standardized p53 grading system used at MSH, the average p53 staining grade for SC and epithelioma was calculated to be 2.3 ± 0.7 and 1 ± 0.7 , respectively ($p=0.002$). An outpatient cohort revealed that 65% of SC was found on the lower eyelid and 84% of BCC was on the upper eyelid. The most common histologic features seen in SC were nuclear atypia (61%) and cytoplasmic vacuoles (55%).

Conclusions: This study provides the first comprehensive staining profile of p53 in benign and malignant sebaceous tissues. We also report for the first time that membrane p53 staining was helpful and appeared to be highly specific for identifying sebaceous hyperplasia. p53 staining was also helpful for identifying SC and intraepithelial spreading tumor cells. The clinical and histologic characteristics of specific eyelid lesions in an outpatient setting are also shared. Lastly, this work incorporates the latest research to synthesize IHC recommendations for improving SC detection.

Keywords: Ocular oncology; Eyelid cancer; p53 tumor suppressor; Sebaceous carcinoma

Introduction

First described by Fuchs in 1878, sebaceous carcinoma (SC) is a unique eyelid malignancy that mimics many other orbital and adnexal lesions clinically and histopathologically [1]. Delayed diagnoses with this aggressive epithelial cancer are common and lead to fatal outcomes; many considering it to be the most lethal of all ocular adnexal tumors with mortality rates reported up to 50% [2-5]. Recent efforts have increased clinical awareness and resulted in earlier SC detection; however the ongoing challenge remains to understand the genes and molecular pathways responsible for SC. Reflecting these efforts, the latest reports have showed decreased mortality rates, ranging from 2%-10%, and identified numerous mutated genes in SC to further study, including *TP53* (OMIM no. 191117) [2,6]. With the

highest number of mutations compared to other genes, the tumor suppressor p53 has also been demonstrated to be an effective IHC marker for differentiating sebaceous carcinoma from basal and squamous cell tissues in addition to being able to map intraepithelial tumor cells [7-10].

Although variable ranges for the incidence of SC have been reported depending on the ethnic population and geographic location, this cancer comprises approximately 5% of eyelid malignancies in the USA making it the second most common eyelid malignancy after basal-cell carcinoma (BCC, approximately 90%) and ahead of squamous-cell carcinoma (SCC, approximately 4%) [2,11-15]. In Asia, the frequency of SC is significantly higher, ranging from 10.2% to 39% [10]. The greater percentage for SC compared to SCC has only recently been established-likely due to an increasing clinical awareness and improvement in staining marker sensitivity. In fact, the largest pathologic series from Europe for eyelid lesions is now outdated regarding the incidence of SC compared to SCC [16]. While sebaceous

glands are present throughout the body, they are most abundant in the ocular region including: tarsal plate meibomian glands, Zeis glands with the cilia, and other eyelid skin. As such, it's not surprising that approximately 75% of diagnosed SCs are periorbital [17-19].

Until recently, limited studies characterizing this tumor were accessible due to less than 300 case-reports having been published [20]. A sentinel publication for understanding the natural history of SC occurred when investigators retrospectively analyzed 1,349 patients with this malignancy from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database [20]. These results represent the largest number of SC samples analyzed to date in a single study and clarified many conflicting reports related to studies about SC using smaller cohorts. This report clarified risk factors for developing SC when looking at skin color, sex, age, and locations and showed they were skewed towards whites (86.2%), men (54%), elderly (mean age at diagnosis as 72), and at the eyelid and/or skin of the face/head/neck, respectively.

Clinical features

Periorbital SC is notoriously reported to mimic a wide spectrum of benign and malignant diseases. These include chalazia, pyogenic granulomas, granulomatous inflammation, papilloma, blepharoconjunctivitis, SCC, BCC, cutaneous horns, sarcoidosis, ocular pemphigoid, etc [21-23]. Shields et al. showed that patients who were eventually diagnosed with SC were initially suspected of having SC after clinical examination in only 32% of cases [6]. Generally, the most commonly reported clinical presentation is either a thickening of the eyelid or a firm yet slowly enlarging pink to yellow-red nodule of the upper eyelid that is resistant to basic chalazia treatments [13,24,25]. The wide range of presentations without any unifying features makes SC detection and screening criteria difficult. Initial clinical suspicion is critical in allowing for the appropriate workup that requires specific markers and preferably frozen sections to preserve intracellular lipid from washout during the preparation process otherwise. Although no consensus for surgical margins has been established, Dogru et al. suggested at least 5 mm of negative surgical margins should be achieved for eyelid SC, with a low threshold for performing additional wide margin biopsies to evaluate for pagetoid spread [26].

Prognosis for SC is poorest in cases with minimal sebaceous differentiation or pagetoid changes, with mortality rates second only to melanoma [5,27]. Mortality rates for primary SC range from 9%-40%, while metastatic SC ranges from 50-67% [2]. While recent reports suggest reduced mortality rates, these advances are reflective of improved surveillance, earlier detection, closer follow-up, and a better understanding of SC's pathogenesis and its related syndromes [2]. Despite ongoing progress, due to the wide myriad of clinical presentations, the time required from disease onset to SC diagnosis is still reported from 1-3 years [13]. Six months has been proposed as the window within which a tissue diagnosis should be made, as a 2-6 fold increase in mortality has been reported for tumors that go undiagnosed beyond this period [5,27,28].

Histopathologic features

A diagnosis of SC is typically made through histological examination of a biopsy specimen. The histological diagnosis of SC is predicated by two major features: 1) the presence of focal sebaceous differentiation and 2) sufficient atypia to warrant a diagnosis of malignancy. Similar to the clinical scenario, SC also remains a

challenging cancer to detect histologically. Masses eventually diagnosed as SC were initially only suspected of being SC after histological examination in 50% of cases [6,14,15]. This difficulty occurs for two reasons: the tumor often presents as an undifferentiated neoplasm from which is difficult to ascertain sebaceous features, and when sebaceous differentiation is overt or clearly evident, SC is difficult to distinguish from other types of sebaceous tissues such as sebaceous adenoma or sebaceous epithelioma [15]. These results demonstrate the need for improving SC detection histologically, as at least half of the SC diagnoses are currently missed histologically.

The atypia seen in sebaceous carcinomas can be nuclear, cytologic, architectural, or a mixture. The sebocytes in the tumor can have enlarged hyperchromatic nuclei, prominent nucleoli, and can show mitotic figures. Often the tumor has an irregularly, poorly defined silhouette that can infiltrate the dermis, subcutis, or even skeletal muscle. A problem with these descriptions is that to a certain extent both sebaceous adenoma and sebaceous epitheliomas may show these changes as well. Also, pagetoid infiltration and vacuolated cytoplasm are often characteristic of SC. Pagetoid spread is a histological observation describing intraepithelial tumor cells that can also be seen in melanoma and squamous cell carcinoma in situ.

Histopathologically, SC is often confused with a spectrum of more benign lesions, such as sebaceous adenoma, sebaceous epithelioma (also commonly referred to as sebaceoma), and basal cell carcinoma with sebaceous differentiation [29]. It is not entirely clear whether transformation from healthy tissue to SC occurs in a staged transformation through one or more of these less malignant lesions, or if these represent related but distinct disease processes. While these mutations are not necessarily a prognostic indicator, they identify novel markers that can be employed through IHC. Indeed, IHC markers like BerEP4, adipophilin, epithelial membrane antigen (EMA), and androgen receptor (AR) have been useful in differentiating SC from SCC and BCC [10]. Of note, adipophilin has been shown capable of identifying intracellular lipid in formalin-fixed, paraffin sections of eyelid SC, obviating the need to use oil red O staining in frozen sections.

Genetic features

Aiding in differentiating benign and malignant sebaceous tissues using genetic information, profiling of SC tissues has found different mutations in tumor suppressors and oncogenes, including: p53, Rad51, K-Ras, and AR [30-33]. Particularly interesting, recent studies may suggest that p53 allow for a superior assessment of behavior and grade for different sebaceous tissues [8,34,35]. At a genetic level, Kiyosaki et al. showed in a subset of 15 SCs from the eyelid that 67% of the cases showed point mutations in *TP53* [7]. In contrast to squamous cell carcinoma, the p53 point mutations were not double based tandem mutations that are typically caused with UV exposure, suggesting a novel UV independent process operating in SC. While similar frequencies of p53 mutations have been found in both low-grade and high-stage SC, no significant correlation has been established between the severity of p53 mutation and tumor size, recurrence, metastasis, pagetoid spread, or location [36]. Recent work by Shalin et al. has shown significant differences in staining between malignant SC and benign lesions [37]. Similarly demonstrating the genetic to tumor association, a report of a patient with Li-Fraumeni syndrome-harboring a germ-line TP53 mutation-was recently reported to have SC [38]. While the implications for mutations in p53 are important in SC, although currently unclear, examining the p53 staining profile in

malignant SC tissues has been helpful in identifying pagetoid spreading cells [32,39]. The nuclei of tumor cells are typically round with granular or smudgy chromatin with small punctate nucleoli. These cells tended to have increased p53 staining patterns, with p53 staining being able to identify residual intraepithelial tumor cells [8,37].

To clarify the role of p53 beyond SC, we chose to characterize p53 staining in both benign and malignant sebaceous tissues. We designed a study to detect and compare p53 expression profiles in different sebaceous tissues. In addition, we report the clinical features of 102 patients with a histopathologic diagnosis of SC from MSH.

Materials and Methods

Acquisition of data

This research was approved for exemption by the Institutional Review Board (IRB) at Mount Sinai Hospital in New York, NY (HS# 13-00222). We performed a retrospective chart review of 102 patients with a confirmed histopathologic diagnosis of SC diagnosed since 1994 to 2012. Repeat immunohistochemistry was performed on 30 cases of SC, 4 cases of sebaceous hyperplasia, 3 cases of sebaceous adenoma, and 4 cases of sebaceous epithelioma. A separate outpatient cohort was used to report the histologic features of 31 cases of SC. This same outpatient cohort was also used to compare the clinical features of 20 cases of SC, 25 cases of BCC, and 21 cases of chalazia SC. The majority of these patients were seen on a referral basis, and therefore, duration of presentation was not available. Patients were managed by either surgical excision or Mohs surgery after preliminary biopsy. All outpatient cases were identified by querying an existing dermatopathology database at MSH, and available chart information was gathered as needed, including demographics, provider, size of tumor, depth of invasion, treatment method, pathological stains, margins, and follow-up information. Disease specific survival data was not available.

Sample collection and pathologic analysis

All SC samples from patients were prepared on slides using standard H&E and immunohistochemical protocols at the Mount Sinai Hospital's Department of Pathology. Samples to be stained for p53 were subjected using an anti-p53 (sc-DO1) polyclonal antibody.

Statistical analysis

Statistical analysis was performed using SPSS (Release 18.0.0, PASW Statistics 18, Polar Engineering and Consulting). Comparison of means between paired groups was done using the independent sample t-test. The cutoff for p-value significance was p less than 0.05.

Results

p53 Staining in benign and malignant sebaceous tissues

A cohort of 102 patients with histopathologic diagnosis of sebaceous carcinoma since 1994 was selected for this analysis. The mean age of diagnosis was 69 years. The majority of patients were male (58%), and 91% of SCs were localized to the eyelid and skin of the head/neck/face. Of all SCs in our cohort, 59% were periorbital/orbital and the remaining 41% included lesions on the forehead, cheek, nose, scalp and trunk.

In order to characterize the expression pattern of p53 in a variety of sebaceous tissues, a complete range from normal to cancerous sebaceous samples from MSH were stained and analyzed by H&E and p53 staining (Figures 1 and 2). When comparing the H&E stained epidermal/dermal layers of normal sebaceous glands to SC, cases of SC showed hyperchromatic, vacuolated, and enlarged nuclei with variable underlying architectural abnormalities. As indicated by yellow arrows, an example of pagetoid spreading with intraepithelial carcinoma tumor can be seen in Figure 1. Additionally, while no detectable p53 was noted in sebocytes from normal sebaceous glands, positive nuclear p53 staining was often observed in pagetoid spreading cells. It is difficult to provide a staining control, however, CK7 was provided as a reference although reports have previously stated differential staining patterns for this marker in sebaceous tissues (Supplementary Figure 1).

In contrast to SC, epitheliomas displayed low numbers of sebocytes with any positive p53 nuclear staining, generally without hyper-expression (Figure 2). SC cells showed strong nuclear p53 staining with hyper-expression, only occasionally with positive cytoplasmic staining. Interestingly, although no nuclear or cytoplasmic p53 expression was observed, membrane p53 staining without atypia was reproducibly observed in all cases of sebaceous hyperplasia. As can be seen in Figure 2 with the blue arrow, the sebocytic cell borders are outlined with positive brown staining which is distinct from pink non-sebocytic staining at the border (Figure 2).

	p53 Nuclear or Cytoplasmic Staining
G1	0-33%
G2	34-66%
G3	67-100%

Table 1: Numerical grading system for p53 staining at MSH.

Using a similar grading system that is used for cancerous tissues at MSH, when the percentage of p53 positive (cytoplasmic or nuclear) staining sebocytes (1=0-33%, 2=34%-66%, 3=67%-100%) (Table 1) in carcinoma samples were quantified, the average grade for SC was calculated to be 2.3 ± 0.7 while the average grade for epithelioma was 1 ± 0.7 ($p=0.002$). A comprehensive p53 grading profile can be seen in Supplementary Figure 2.

Clinical features of outpatient sebaceous carcinoma compared to bcc and chalazia

Eyelid SC is especially well known for its ability to simulate many more benign lesions (Figure 3). To examine commonly misdiagnosed eyelid lesions in an outpatient setting, the clinical features from outpatient charts were collected, including 20 eyelid SC, 25 eyelid BCC, and 21 eyelid chalazia. The average age of patients diagnosed with eyelid SC, BCC, and chalazia was 73, 71 and 38 years, respectively (Table 2). Female patients accounted for 60%, 92%, and 33% for SC, BCC, and chalazia respectively. There was no statistical difference between right and left eye involvement. 65% of SC occurred on the upper eyelid. In this cohort, lower eyelid involvement was associated with BCC when compared to SC ($p<0.001$) and chalazia independently ($p=0.001$).

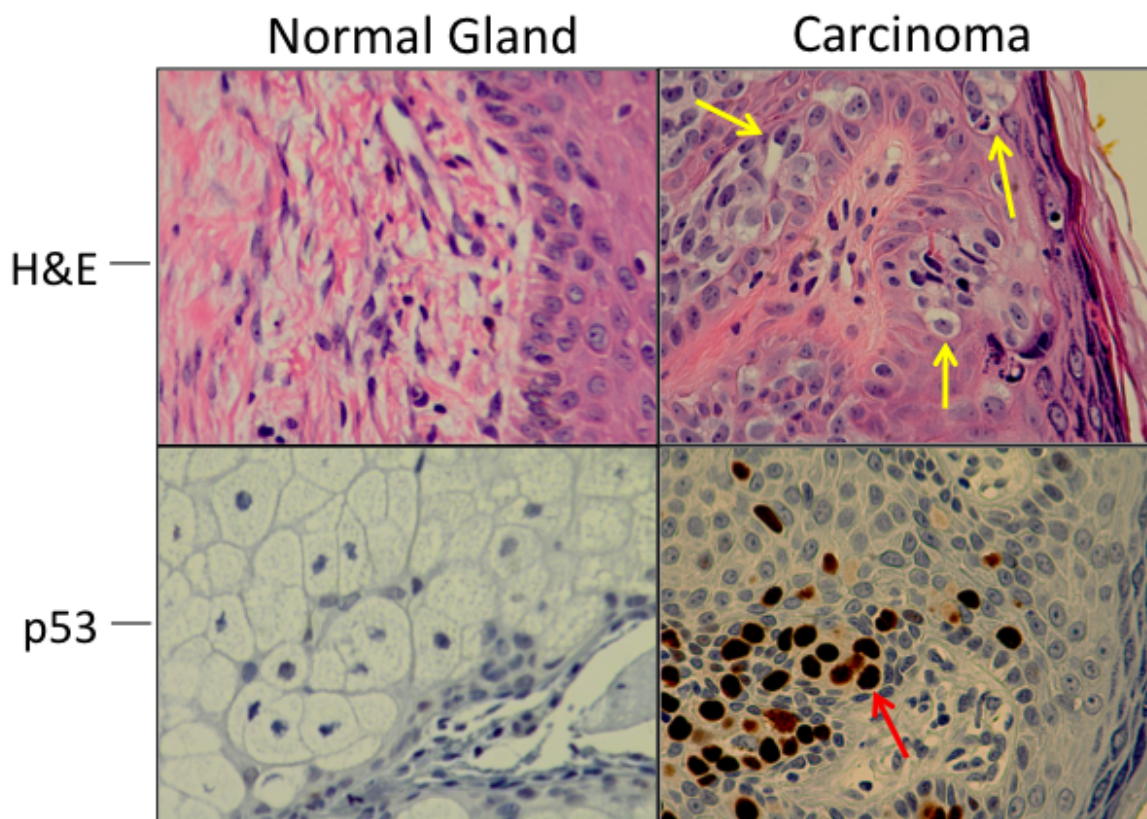
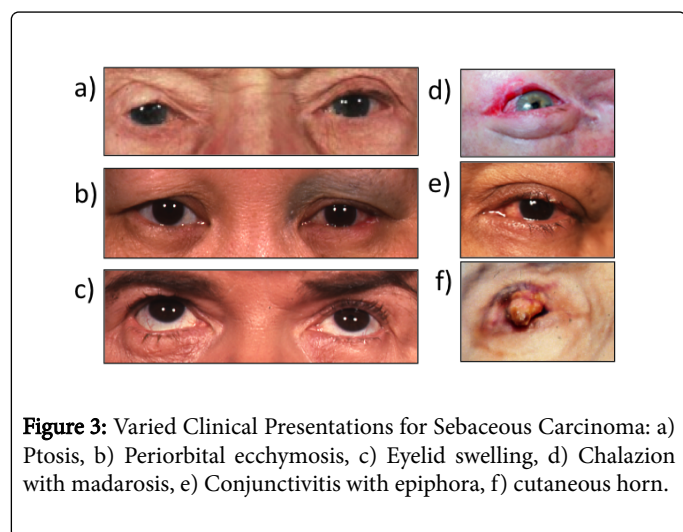
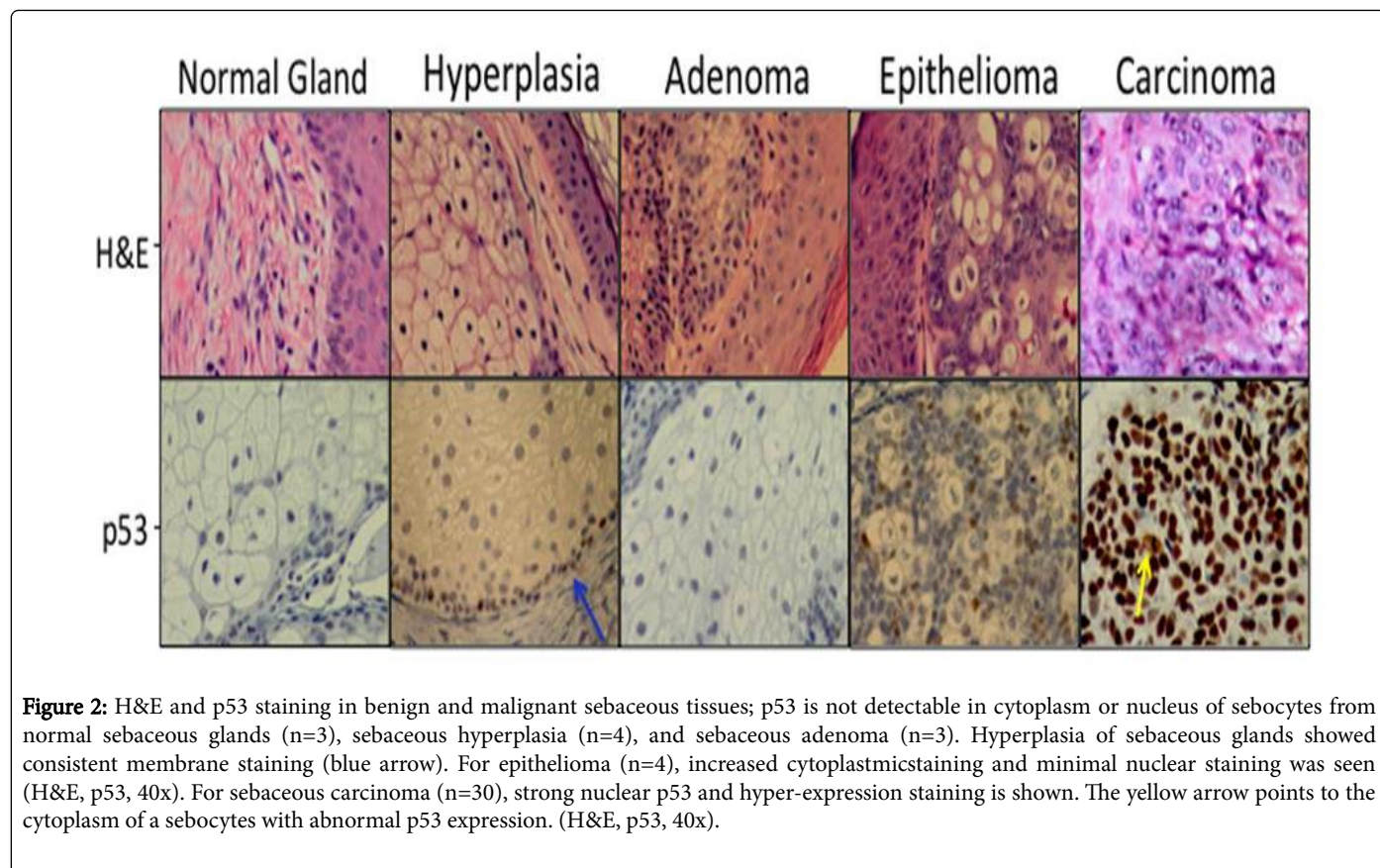


Figure 1: Expression patterns for p53 in normal sebaceous gland and carcinoma with pagetoid spread; H&E and p53 staining comparing normal sebaceous gland (left side) to SC (n=30) with pagetoid spread (right side). Normal sebaceous glands show no abnormality in architecture with sebocytes containing vacuolated cytoplasm that do not stain positive for p53. Intraepithelial tumor cells (pagetoid spread) can be seen with SC by H&E (upper right, yellow arrows), which often stain positive for p53 (lower right, red arrow).

	Sebaceous Cell Carcinoma (n=20)	Basal Cell Carcinoma (n=25)	Chalazia (n=21)
Average Age (yrs)	72.8	71.3	37.8
Male (%)	40.0%	8.0%	66.7%
Female (%)	60.0%	92.0%	33.3%
Average Size (mm)	8.75	3.56	6.62
Right Eye Involvement (%)	65.0%	40.0%	47.6%
Left Eye Involvement (%)	35.0%	60.0%	57.1%
Lower Lid Involvement (%)	35.0%	84.0%	42.9%
Upper Lid Involvement (%)	65.0%	8.0%	61.9%

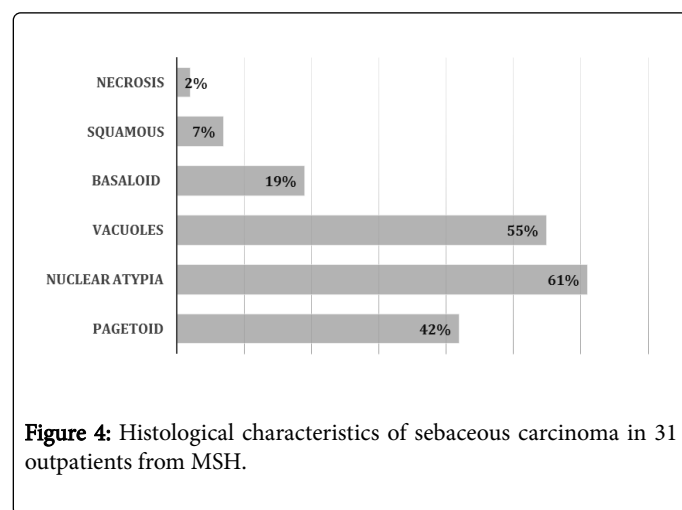
Other Eye Location Involvement (%)	15.0%	8.0%	0.0%
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Table 2: Clinical features of MSH patients with SC compared to BCC and chalazia.



Thirty-one histopathologic descriptions of SC were available from this outpatient cohort (Figure 4). Approximately 58% of these patients were surgically operated on by oculoplastic surgeons. The average size of SC was 8.8 mm. Of these, 13 of 31 specimens (42%) specifically noted pagetoid spread of the tumor. Nineteen specimens (61%) noted

nuclear atypia, and 17 specimens (55%) noted vacuoles in the cytoplasm (Figure 4). Of note, 6 specimens (19%) noted basaloid cell features and 2 (7%) noted squamous cell features. Necrosis was noted in 2 samples (7%). The recurrence rate for SC in this patient cohort was 15% (3 of 20 patients).



Discussion

Ongoing research continues to unravel the molecular and genetic aberrations responsible for SC. Previous studies have attempted to differentiate sebaceous carcinoma from benign lesions using multiple staining markers including anti-cytokeratin, mismatch repair genes, cytokines, adipophilin, p53 and mitotic figures [12,31,33,34,40]. These staining algorithms can be complicated and expensive, but a single marker that is consistently predictive of SC versus less dangerous lesions remains elusive.

Part of this problem is that it is still unclear whether development of sebaceous cell carcinoma represents a malignant progression from more benign lesions or a distinct disease process [13,14,20,32]. It is likely that many mutations that may result in benign lesions, result put those cells at risk of SC given certain key mutations. Mismatch repair genes that result in microsatellite instability have been established as one known mechanism, but only capture a subset of patients, and is more likely to be seen in patients with Muir-Torre syndrome (MTS) [41-43]. Indeed, patients with eyelid sebaceous carcinoma have been reported in immunocompromised patients with MTS [44].

The present study is in general agreement with work by Jakobiec, Kiyosaki, and Shalin [7,8,37]. Strong nuclear p53 staining was observed in SC in contrast to the sebaceous hyperplasia, sebaceous adenomas, and epithelioma. Newly reported in this study is the utility of p53 to differentiate among benign lesions. Epithelioma consistently showed a mild p53 positivity to differentiate it from SC (p=0.002). Additionally, membrane p53 staining was a consistent finding in sebaceous hyperplasia, an observation not previously reported to date. Therefore, our data suggests that staining with p53 can help in differentiating among both benign and malignant sebaceous diagnoses. Additionally, our data showing pagetoid spread as being identified by nuclear p53 staining validates that p53 may be helpful for assessing tumor extension [8].

Despite increasing ability to differentiate histologically SC from similar appearing benign lesions, the ability to differentiate clinically still presents a significant challenge [2,3,6,13-15,23,25,27,38,45]. The ability to mimic benign lesions such as BCC and chalazia are especially pronounced on the eyelid and it becomes critical to include SC in the differential diagnosis of any ocular disease including benign appearing masses and inflammatory conditions. The small cohort presented represents the first known direct comparison between these three diagnoses. In general, SC and BCC were found in older individuals, while chalazia were more often found in younger adults. Immunohistochemistry, using a panel of markers can be used to distinguish SC from both basal cell carcinoma and squamous cell carcinomas. SC usually stain positive for EMA and cyokeratin 7 (CK7) and negative for BER-Ep4. Adipophilin, a lipid marker, is a very sensitive marker for confirming sebocytic differentiation [46].

BCC is well known to have lower eyelid predominance [47]. The lack of lower lid predominance of SC in this cohort is consistent with this lesion evolving independently of UV radiation, as suggested by Kiosaki [7]. Thus it may be helpful to clinicians to have a high suspicion for SC in patients without a history of UV exposure, in addition to any immunocompromised state [44]. This study was limited by the lack of p53 mutation sequencing, which may have been able to correlate mutational status with SC progression, pagetoid spreading potential, or other immunohistochemical or clinical parameters. Other investigators have identified multiple genes including p53, RB1 and PIK3CA could be the drivers in ocular

sebaceous carcinomas and mismatch repair mutations could be the drives in extraocular sebaceous carcinoma [48].

Conclusion

SC is a dangerous eyelid cancer with significant mimicry potential clinically and histopathologically. We recommend early referral to an oculoplastic surgeon for all suspicious eyelid cases and p53 staining as a useful adjunct to H&E staining for all eyelid biopsies. Staining patterns and localization for p53 will be helpful in differentiating SC from other malignancies and also between different sebaceous tissues; in particular, membrane p53 staining may be helpful in identifying sebaceous hyperplasia.

Acknowledgements

We are grateful to Dr. Murray Meltzer (Department of Ophthalmology,) for providing his clinical insight and SC photograph library. We are also grateful to Patricia Siegler, Department of Pathology Staff, and Dermatopathology Staff at MSH for their valuable contributions to the clinical and histology sections in this manuscript. We would also like to thank Dr. Pierre-Jacques Hamard (Department of Oncological Sciences) for his conceptual input related to p53.

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