

The Role of α -Smooth Muscle Actin in Confirming the Microinvasion of Laryngeal Squamous Cell Carcinoma

Yuping Bai

Beijing Tongren Hospital

Changli Yue

Beijing Tongren Hospital

Zhichun Lu

Boston University Medical Center: Boston Medical Center

Honggang Liu (✉ liuhg1125@163.com)

Beijing Tongren Hospital

Research

Keywords: α -Smooth muscle actin, Microinvasive laryngeal squamous cell carcinoma, Histomorphologic feature, Pathological diagnosis, Carcinoma-associated fibroblasts

Posted Date: November 24th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-112929/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

The diagnosis of microinvasive laryngeal squamous cell carcinoma (mLSCC) is not always straightforward and sometimes can be very challenge in daily clinical practice, particularly in the circumstances with differentials such as SIL with inverted growth pattern and PEH. The focus of the diagnosis is the confirmation of microinvasion. Cancer-associated fibroblasts (CAFs), as the major element of tumor microenvironment, are believed to participate actively in the invasion of tumor cells. In this study, we sought to evaluate the diagnostic role of α -SMA labelling CAFs in mLSCC, with comparison of laryngeal squamous intraepithelial lesion (SIL) and benign pseudoepitheliomatous hyperplasia (PEH).

Methods

A total of 81 laryngeal biopsy specimens were retrieved, including 41 cases of mLSCC with depth of invasion no more than 3 mm, 20 laryngeal SIL, and 20 PEH. All tissues were stained for immunohistochemistry, using antibody against the α -SMA antigen. We observed the presence of α -SMA positive fibroblasts in mLSCC, compared the results with that of laryngeal SIL and benign PEH by Chi-square statistics test, and investigated the correlation between tumor histological characteristics and the presence of CAFs in mLSCC.

Results

Immunoreactivity of α -SMA was detected in twenty-nine mLSCC (29/41, 70.7%), while no reactivity was observed in laryngeal SIL (0/20, 0%), and few in PEH (2/20, 10%). The α -SMA expression pattern in stromal fibroblasts of mLSCC were significantly different from those of SIL ($\chi^2 = 26.966$, $p=0.000$) and PEH ($\chi^2 = 19.838$, $p=0.000$). This support that, α -SMA as an immunolabeling of CAFs plays a reliable role in confirming the microinvasion of LSCC. In addition , we finds that there is dense lymphoplasmacytic infiltration in the stroma of a few mLSCC (7/41, 17.1%) cases rather than tumor-related desmoplastic hyperplasia. And it seems that there is a negative correlation between dense lymphoplasmacytic infiltration and the presence of CAFs in LSCC.

Conclusions

Our study highlights practical role of utilizing α -SMA in the diagnosis of microinvasive laryngeal squamous cell carcinoma, with emphasis on variable histomorphologic features of mLSCCs.

1. Introduction

As a common malignant otorhinolaryngologic neoplasm, laryngeal cancer accounts for 90% of malignancies occurring in head and neck region [1, 2]. Of such, squamous cell carcinoma is the most common neoplasm accounting for more than 95% of all the laryngeal carcinoma [1, 3]. The prognosis of advanced laryngeal cancer is currently not optimal [4]. However, the diagnosis of mLSCC is not always

straightforward, particularly in the circumstances with differentials such as SIL with inverted growth pattern and PEH.

MLSCC is defined as LSCC that have penetrated the basement membrane and infiltrated into the superficial compartment of the lamina propria [1, 3]. Currently, the confirmation for microinvasion of LSCC is mainly based on constellate histomorphologic features, including tumor cells penetrating basement membrane of the surface epithelium, the downward growth of tumor islands, cords, or isolated tumor cells, with associated desmoplastic stromal changes, excessive deposition of the extracellular matrix, and neovascularization. However, the distinct basement membrane border is usually not clear, the invasive carcinomas may be extremely well-differentiated, and there are no clear-cut criteria for tumor-related desmoplasia.

Previous studies on the pathological diagnosis of LSCC always focus on the tumor cells [5, 6], we sought to explore the diagnosis and differential diagnosis of mLSCC from tumor stroma. Fibroblasts activated in the tumor microenvironment, named as myofibroblasts, peritumoral fibroblasts or cancer-associated fibroblasts (CAFs), has been found typically expressing α -SMA and sharing some characteristics of fibroblasts and smooth muscle cells [7]. As a major component of tumor stroma, CAFs are believed to play a major role in tumor growth, invasion, and metastasis [8-10].

Our hypothesis is that α -SMA can be used as an ancillary biomarker to help with rendering definite diagnosis of mLSCC. In our study, we sought to investigate the presence and expression pattern of α -SMA positive fibroblasts in mLSCC with comparison with H&E in-situ histomorphology, and compare with the results from laryngeal SIL and benign PEH.

2. Materials And Methods

2.1 Study Design and Patient Selection

Specimens were retrieved from the archives of Department of Pathology, Beijing Tongren Hospital, affiliated to Capital Medical University, from March 2018 to January 2020. A total of eighty-one laryngeal biopsy samples from patients of underwent laryngeal mass evaluation, which included 41 cases of mLSCC (Group 1), 20 cases of laryngeal SIL(Group 2), and 20 cases of laryngeal PEH (Group 3). The depth of invasion of mLSCC were not greater than 3 mm. Cases of SIL (WHO 2017) including lesions of low-grade, high-grade and carcinoma in situ. Cases of PEH occurred in lesions including simple or chronic inflammatory hyperplasia, tuberculous granulomatous inflammation, keratosis and polyps of larynx. Additional 5 biopsy of normal adult laryngeal mucosa used as negative controls. All cases were independently reviewed by Three senior pathologists (YB, CY and HL), and any cases with diagnostic discordance were excluded. A representative section was selected for immunohistochemical staining. Clinical data collected includes age, gender, and tumor laterality.

Based on the depth of invasion (DOI), the 41 samples of mLSCC were divided into three subgroups. Group 1-1 DOI ≤ 1 mm, Group 1-2 DOI more than 1mm but ≤ 2 mm, Group 1-3 DOI more than 2 mm but

≤3 mm. The depths of the invasion were measured from the basement membrane of the epithelium to the deepest point of invasion of the tumor by using microscope rulers.

2.2. Histological Evaluation and Immunohistochemical staining

Formalin-fixed paraffin-embedded tissues were sectioned (3 µm), and stained for immunohistochemistry, using antibody against the α-SMA antigen (clone UMAB237, OriGene) and Peroxidase-Streptavidin method. Positive and negative controls gave appropriate results for each procedure. The presence of brownish-yellow cytoplasmic staining was considered to be a positive result. Three-tier system has been used to quantitative evaluate the immunoreactivities. More than 50% of fibroblasts positive for α-SMA were considered as diffusely positive, between 10-50% of positive fibroblasts were considered as focal positive, and less than 10% positive fibroblasts were rare or scattered positive. Positive endothelial cells were counted as positive internal controls and excluded from the analysis.

2.3. Statistical Analysis

Chi-square statistics test was used to compare the expression pattern of α-SMA in stromal fibroblasts. Statistical software SPSS 23.0 was used for statistical analysis. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Clinical features

Among the 41 cases of mLSCC, 39 cases were men (39/41, 95.1%) and 2 cases were women (2/41, 4.9%). Patients' age ranged from 44 to 81 years (mean 60 years). There were 35 cases occurred in the vocal cords (35/41, 85.4%), of which 20 cases in the right vocal cords, and 15 cases in the left vocal cords. The remaining 6 cases occurred in other parts of the larynx, including 3 cases at anterior joint, 2 cases at right pseudo-vocal cord, and 1 case at right arytenoid zone. (Table 1)

3.2 Histologic Features and Immunohistochemical results

Grossly, 36 of 41 mLSCC were flat/non exophytic type, and 5 cases were exophytic papillary or verrucous type. Microscopically, 29 cases showed an infiltrative growth pattern, which were characterized by small irregular tumor nests, cords and/ or single tumor cells with poorly defined infiltrating borders, while 12 cases showed an expansive growth pattern, which were characterized by large tumor islands with well-defined pushing borders. Varying degrees of desmoplastic stromal reaction were detected in 21 cases. 7 cases showed densely chronic inflammatory infiltrates with no definite desmoplastic reaction identified, and 13 cases showed no distinct stromal changes. (Table 2)

Of the 41 mLSCC cases, 29 cases demonstrated α-SMA positivity in the stroma (29/41, 70.7%) (Fig. 1 A-C, Fig. 2 A-C), of which, 22 cases were diffuse positive, 5 were focally positive and 2 were scattered positive. And 12 of 41 mLSCCs were stromal α-SMA negative (12/41, 29.3%). The correspondence between the

histological features and α -SMA expression of mLSCC were shown in Table 2. Of the 29 α -SMA positive cases of mLSCC, there were 4 cases in Group 1-1, 9 cases in Group 1-2, and 16 cases in Group 1-3. The positive rate in Group 1-2 and 1-3 (DOI greater than 1 mm) were significantly higher than that in Group 1-1 (DOI \leq 1 mm) ($\chi^2 = 4.230$, $p=0.040$) (Table 3). Of the 12 cases of α -SMA negative mLSCC, half of them showed densely chronic inflammatory infiltrates (Fig. 3). And the remaining 6 negative cases showed no distinct stromal changes, of which 5 cases belonged to Group 1-1 (DOI \leq 1 mm) and 1 case belonged to Group 1-2 (1 mm \leq DOI \leq 2 mm). In H&E stained sections, the stroma corresponding to α -SMA positive areas in mLSCC were rich in collagen with increased stromal cellularity. The desmoplastic stromal cells /CAFs appeared around the epithelial tumor nests, arranging in streaming patterns.

All cases of SIL didn't show distinct stromal reactions and showed negativity of α -SMA (0/20, 0%) (Fig. 1 D-F). And only 2 of the 20 PEHs were α -SMA positive (2/20, 10%), the remaining 18 cases of PEH all showed α -SMA negativity (Fig.2 D-F). The α -SMA expression pattern in stromal fibroblasts of mLSCC were significantly different from those of SIL ($\chi^2 = 26.966$, $p=0.000$) and PEH ($\chi^2 = 19.838$, $p=0.000$) (Table 4). As to the 2 positive PEH cases, one was a case of vascular vocal cord polyp showed focal positive expression of α -SMA, the other was a case of vocal cord keratosis showed diffuse positive. It should be noted that, the patient of this vocal cord keratosis had undergone surgical resection at the same site 3 months ago, and this time was a postoperative recurrence. Unlike the α -SMA positive fibroblasts in mLSCC appearing around the epithelial tumor nests, the distribution of immunostained positive fibroblasts against α -SMA in PEH seems to have nothing to do with the downward growing epithelial lesions. The SMA-positive fibroblasts in PEH also appeared in lamina propria covered with flat epithelium and interstitium away from epithelium. While the fibroblasts around the down-growing epithelial nests in PEH were not always SMA positive, or even completely negative in some area.

4. Discussion

In our study, mLSCC were most often occurred in men, mainly in elders. The vocal cord was the most common site with right predominancy. Grossly, mLSCC were mainly flat with a few cases showing exophytic or verrucous papillary configurations. Histomorphologically, the growth pattern at the invasive front of mLSCC is mainly infiltrative, but the expansive growth pattern are not uncommon. Three types of tumor associated stromal changes were observed in our study, typical desmoplastic stromal changes, densely chronic inflammatory infiltrates, and no distinct stromal changes assembling adjacent normal lamina propria. Among these, desmoplastic reaction is the most common feature observed in mLSCC.

As the major component of tumor microenvironment, CAFs are found to be present in a variety of tumors such as esophageal cancer, lung cancer, hepatocellular carcinoma, and kidney cancer. Recent studies showed that the detection of CAFs can aid in the diagnosis of microinvasive ductal carcinoma, endoscopically removed invasive colorectal adenocarcinoma, and assist in differentiating between pancreatic ductal adenocarcinoma and chronic pancreatitis [11-13]. Study from Kumar et al and New et al using a mouse xenograft model of head and neck squamous cell carcinoma demonstrated CAFs play a significant role in invasion and the progression of squamous cell carcinoma [14, 15]. In our study,

majority of mLSCC (29/41, 70.7%) showed positive reactivity of α -SMA on CAFs. In contrast, there was no or few immunoreactivity identified in laryngeal SIL and PEH (0/20, 0% ; 2/20, 10%). SIL and PEH are lesions that often need to be differentiated from mLSCC. Although it is said that SIL showed an intact basement membrane, but this is difficult to identify histologically. SIL with inverted growth pattern or tangential section can also be difficult to distinguish from invasive carcinoma. PEH, a not uncommon benign lesion occurring in the laryngeal mucosa, can be seen in chronic irritation, non-specific inflammation, tumors such as granular cell tumor, laryngeal tuberculosis and fungal infections, or keratosis [16-18]. As the name PEH implies, the histomorphologic appearance assemble to invasive carcinoma and characterized with mucosal epithelial hyperplasia showing sub-epithelial extension in lamina propria. The irregular epithelial projections in PEH is difficult to distinguish from the invasive nests of well-differentiated squamous cell carcinoma. In our study, the α -SMA expression pattern in stromal fibroblasts of mLSCC were significantly different both from those of SIL and PEH. This support that, the detection of CAFs by immunohistochemistry against α -SMA plays a valuable role in confirmatory diagnosis of mLSCC, and would be used as a reliable marker for the diagnosis of mLSCC and differentiated from SIL and PEH.

The origin of CAFs has been under debate for a long time. Several hypotheses have been proposed for the origin of CAFs such as resident tissue fibroblasts, bone marrow-derived mesenchymal stem cells, or epithelial cells [19]. A commonly accepted theory for CAFs origin points to resident tissue fibroblasts, which are activated by TGF- β 1 and converted into CAFs [20]. TGF- β 1, mainly localized in exosomes released by cancer cells, can promote the proliferation and expression of CAF markers [21]. Then CAFs secrete various cytokines, chemokines and inflammatory mediators such as stromal cell-derived factor 1 (SDF-1/CXCL12) influence the growth and invasion of tumor [22]. This can explain why CAFs can be widely detected by α -SMA in mLSCC but rarely in laryngeal SIL and PEH. As for the two cases of PEH with SMA positivity in the interstitium, we believe that this is due to interstitial myofibroblasts transformation caused by fibrosis. Fibrosis in vascular vocal cord polyps seems not uncommon. As for the positive case of keratosis, the patient had undergone surgical resection months ago, and the fibrosis should be the repair for injury caused by the previous surgery. Different from the SMA positive fibroblasts in mLSCC appearing around the invasive epithelial tumor nests, positive cells in PEH can appear in lamina propria covered with flat epithelium or in the interstitium away from epithelium. And fibroblasts surrounding the downward growing epithelial nests in PEH may be SMA negative. The different distribution characteristics of SMA -positive fibroblasts can be used to distinguish CAFs from myofibroblasts in fibrosis. The driving force of myofibroblast differentiation in fibrosis are mainly mechanical tension and TGF- β 1 which is released from a variety of inflammatory cells and platelets in the microenvironment of damaged or fibrotic tissue [23].

Comparing the histomorphologic features with the results of immunohistochemical staining of mLSCC, it was found that α -SMA positive cases, especially diffusely positive cases, were concentrated in cases showed flat/non-exophytic gross appearance, infiltrative growth pattern, or with desmoplastic stromal reaction in the stroma. While cases showed exophytic gross appearance, expansive growth pattern or dense lymphoplasmacytic infiltration in the stroma tend to be focal, scattered positive or negative.

Especially the interstitial dense lymphoplasmacytic infiltration, our study support that there is a negative correlation between this characteristics and the presence of CAFs in LSCC. Similar findings were reported by Zidar N et al [24]. In our study, six of 7 cases of mLSCC with dense lymphoplasmacytic infiltration were stromal negative for α -SMA, and the remaining 1 case was focally positive. Furthermore, 5 of these 6 cases with α -SMA negative labelling showed expansive growth pattern at the invasive front. It has been reported that pushing borders and good cohesion of the deep invasive front, and high levels of tumor-infiltrating lymphocytes are indicators of good prognosis for laryngeal squamous cell carcinoma [25, 26]. This may be related to less or no CAFs formation in stroma under these conditions, for it is reported that overexpression of CAFs correlates with poor prognosis in some tumors [27, 28]. However, due to small sample and not enough long term follow-up data available in current study, a larger scale study is needed to provide a further confirmatory.

In our cohort, a small percentage (12/41, 29.3%) of mLSCC showed no immunoreactivity identified with α -SMA in the stroma, 6 of which showed dense lymphoplasmacytic infiltration as we said above, and the remaining 6 showed no distinct stromal changes. While, study from Kojc N et al reported that all cases of LSCC contain α -SMA positive stromal cells [29]. This difference may arise from the variabilities of sampling tissue and cases selection in some degree. There was no information about depth of invasion of the LSCC mentioned in their study. In our cohort, we collected mLSCC with depth of invasion not greater than 3 mm. We speculate that it may be due to the poor stromal response when tumor invades superficially. In our study, we compared positivity rate of α -SMA between cases with DOI less than 1 mm and DOI greater than 1mm, we found that the higher positive frequency of α -SMA was observed in mLSCC with DOI greater than 1 mm ($P < 0.05$). This indicates CAF formation is gradually enhanced along with the depth of tumor invasion. In addition, of the 6 α -SMA negative mLSCC cases showed no distinct histological changes in the stroma, 5 cases belonged to Group 1-1 ($DOI \leq 1$ mm) and 1 case belonged to Group 1-2 ($1 \text{ mm} < DOI \leq 2$ mm). This most likely due to the fact that the depth of tumor invasion is too superficial, resulting in a too weak stromal response to be detected and the tumor morphological characteristics have not yet been shown, which further support our hypothesis.

In summary, mLSCC is predominantly flat/non-exophytic grossly, and often histologically shows an infiltrative growth pattern. Varying degrees of desmoplastic stromal reaction is observed in most cases, but in a few cases it is replaced by dense lymphoplasmacytic infiltration in the stroma. Our study support that there is a negative correlation between dense lymphoplasmacytic infiltration in the stroma and the presence of CAFs in LSCC. Our study further supports α -SMA, as a surrogate marker for CAFs, plays a reliable role in confirmatory invasion for mLSCC.

Abbreviations

mLSCC: microinvasive laryngeal squamous cell carcinoma

CAF: Cancer-associated fibroblasts

SIL: laryngeal squamous intraepithelial lesion

PEH: pseudoepitheliomatous hyperplasia

Declarations

Acknowledgements

We thank Dr Lei Sun from Pathology Department, Beijing Ditan Hospital for his secretary support.

Authors' contributions

YB was a major contributor in writing the manuscript. CY and HL participated in the analysis of histological and the immunohistochemical staining results. ZL and HL participated in the design of the study and helped write the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by Sail Plan of Beijing Municipal Hospital Administration (No. ZY LX201814) to Hg Liu.

Availability of data and materials

All data generated or analyses during this study are included in this published article

Ethics approval and consent to participate

Ethical approval was granted by Ethics Committee of Beijing Tongren Hospital, Capital Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1 Department of Pathology, Beijing Tongren Hospital, Capital Medical University, Beijing Key Laboratory of Head and Neck Molecular Diagnostic Pathology, Beijing, 100730, China

2 Department of Pathology and Laboratory Medicine, Boston Medical Center/Boston University School of Medicine, Boston, MA

References

1. Lester D. R. Thopson BMW. Diagnostic pathology: Head and neck, 1st edn; 2011.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians* 2018, 68(6):394-424.
3. M.Wenig B. Atlas of head and neck pathology, 3rd edn; 2015.
4. Steuer CE, El-Deiry M, Parks JR, Higgins KA, Saba NF. An update on larynx cancer. *CA: a cancer journal for clinicians* 2017, 67(1):31-50.
5. Marioni G, Pillon M, Bertolin A, Staffieri A, Marino F. The role of survivin expression in the differential diagnosis of laryngeal (glottic) verrucous squamous cell carcinoma. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* 2007, **33**(2):229-233.
6. Marioni G, Agostini M, Cappellesso R, Bedin C, Ottaviano G, Marchese-Ragona R, et al: miR-19a and SOCS-1 expression in the differential diagnosis of laryngeal (glottic) verrucous squamous cell carcinoma. *Journal of clinical pathology* 2016, 69(5):415-421.
7. De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. *The Journal of pathology* 2003, 200(4):429-447.
8. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. *Nature* 2001, 411(6835):375-379.
9. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature* 2004, 432(7015):332-337.
10. Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer research* 1999, 59(19):5002-5011.
11. Hua X, Yu L, Huang X, Liao Z, Xian Q. Expression and role of fibroblast activation protein-alpha in microinvasive breast carcinoma. *Diagnostic pathology* 2011, **6**:111.
12. Zhang D, Zhu H, Harpaz N. Overexpression of $\alpha 1$ chain of type XI collagen (COL11A1) aids in the diagnosis of invasive carcinoma in endoscopically removed malignant colorectal polyps. *Pathology, research and practice* 2016, 212(6):545-548.
13. Garcia-Pravia C, Galvan JA, Gutierrez-Corral N, Solar-Garcia L, Garcia-Perez E, Garcia-Ocana M, et al. Overexpression of COL11A1 by cancer-associated fibroblasts: clinical relevance of a stromal marker in pancreatic cancer. *PloS one* 2013, 8(10):e78327.
14. New J, Arnold L, Ananth M, Alvi S, Thornton M, Werner L, et al. Secretory Autophagy in Cancer-Associated Fibroblasts Promotes Head and Neck Cancer Progression and Offers a Novel Therapeutic Target. *Cancer research* 2017, 77(23):6679-6691.
15. Kumar D, New J, Vishwakarma V, Joshi R, Enders J, Lin F, et al. Cancer-Associated Fibroblasts Drive Glycolysis in a Targetable Signaling Loop Implicated in Head and Neck Squamous Cell Carcinoma Progression. *Cancer research* 2018, 78(14):3769-3782.

16. El-Khoury J, Kibbi AG, Abbas O. Mucocutaneous pseudoepitheliomatous hyperplasia: a review. *The American Journal of dermatopathology* 2012, 34(2):165-175.
17. Sproat R, Wong G, Rubin J. Granular Cell Tumour of the Larynx. *Head and neck pathology* 2016, 10(4):538-540.
18. Payne J, Koopmann CF, Jr.. Laryngeal carcinoma—or is it laryngeal blastomycosis. *The Laryngoscope* 1984, 94(5 Pt 1):608-611.
19. Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. Cancer-Associated Fibroblasts: Their Characteristics and Their Roles in Tumor Growth. *Cancers* 2015, 7(4):2443-2458.
20. Rønnov-Jessen L, Petersen OW. Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. *Laboratory investigation; a journal of technical methods and pathology* 1993, 68(6):696-707.
21. Ringuette Goulet C, Bernard G, Tremblay S, Chabaud S, Bolduc S, Pouliot F. Exosomes Induce Fibroblast Differentiation into Cancer-Associated Fibroblasts through TGF β Signaling. *Molecular cancer research : MCR* 2018, 16(7):1196-1204.
22. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005, 121(3):335-348.
23. Darby IA, Zakuan N, Billet F, Desmoulière A. The myofibroblast, a key cell in normal and pathological tissue repair. *Cellular and molecular life sciences : CMLS* 2016, **73**(6):1145-1157.
24. Kojc N, Zidar N, Vodopivec B, Gale N. Expression of CD34, alpha-smooth muscle actin, and transforming growth factor beta1 in squamous intraepithelial lesions and squamous cell carcinoma of the larynx and hypopharynx. *Human pathology* 2005, 36(1):16-21.
25. Bryne M, Jenssen N, Boysen M. Histological grading in the deep invasive front of T1 and T2 glottic squamous cell carcinomas has high prognostic value. *Virchows Archiv : an international journal of pathology* 1995, 427(3):277-281.
26. Spector ME, Bellile E, Amlani L, Zarins K, Smith J, Brenner JC, et al. Prognostic Value of Tumor-Infiltrating Lymphocytes in Head and Neck Squamous Cell Carcinoma. *JAMA otolaryngology– head & neck surgery* 2019, 145(11):1012-1019.
27. Chen J, Yang P, Xiao Y, Zhang Y, Liu J, Xie D, et al. Overexpression of α -sma-positive fibroblasts (CAFs) in Nasopharyngeal Carcinoma Predicts Poor Prognosis. *Journal of Cancer* 2017, 8(18):3897-3902.
28. Dourado MR, Guerra ENS, Salo T, Lambert DW, Coletta RD. Prognostic value of the immunohistochemical detection of cancer-associated fibroblasts in oral cancer: A systematic review and meta-analysis. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* 2018, 47(5):443-453.
29. Zidar N, Gale N, Kambic V, Fischinger J. Proliferation of myofibroblasts in the stroma of epithelial hyperplastic lesions and squamous carcinoma of the larynx. *Oncology* 2002, 62(4):381-385.

Tables

Table 1. Clinical features of mLSCC

Features	No. (%)
Sex	
Male	39 (95.1%)
Female	2 (4.9%)
Age (years)	
Median	60
Range	44-81
Location and laterality	
Vocal Cords	35 (85.4%)
	Right 20
	Left 15
Others*	6 (14.6%)

* Others include 3 at anterior joint, 2 at right pseudo-vocal cord and 1 at right arytenoid zone

Table 2. Correlation of α -SMA expression and histological features of mLSCC

α -SMA expression					
Histological features	Diffuse Positive	Focal Positive	Scattered Positive	Negative	Total No. (%)
Grossly appearance					
Flat protruded or flat	21	5	0	10	36 (87.8%)
Exophytic papillary /verrucous	0	1	2	2	5 (12.2%)
Tumor growth pattern at the invasive front					
Infiltrative pattern	19	1	2	7	29 (70.7%)
Expansive pattern	2	5	0	5	12 (29.3%)
Morphology of the stroma					
Desmoplastic stromal reaction	21	1	0	0	22 (53.6%)
Dense lymphoplasmacytic infiltration	0	1	0	6	7 (17.1%)
No distinct changes*	0	4	2	6	12 (29.3%)
Total No. (%)	22 (53.7%)	5 (12.2%)	2 (4.9%)	12 (29.3%)	41 (100%)

Table 3. Stromal expression of α -SMA in mLSCC with different depth of invasion

Group	Positive No. (%)	Total	χ^2 value*	P value*
Group 1-1	4 (40.0%)	10	4.230	0.040
Group 1-2	9 (69.2%)	13		
Group 1-3	16 (88.9%)	18		

*Group 1-1 versus Group1-2 and Group 1-3

Table 4. Comparison of stromal α -SMA expression between mLSCC, laryngeal SIL and PEH

Group	Positive No. (%)	Total	χ^2 value		P value	
			1	2	1	2
Group1	29 (72.0%)	41	26.966	19.838	0.000	0.000
Group2	0 (0%)	20				
Group3	2 (10%)	20				

χ^2_1 P1 refers to Group1 versus Group2 ;

χ^2_2 P2 refers to Group1 versus Group3 .

Figures

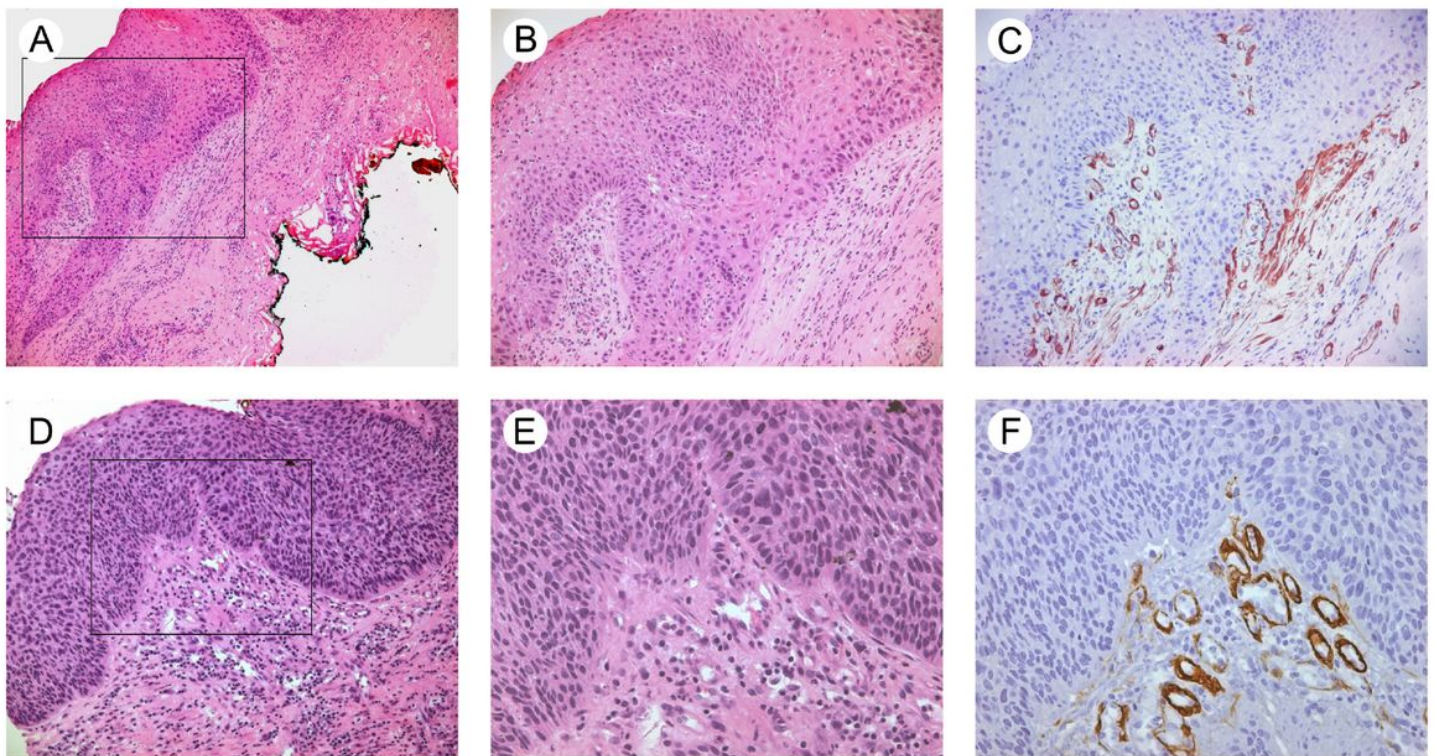


Figure 1

Comparison of stromal α -SMA expression in mLSCC (A-C) and laryngeal SIL (D-F). B and E show the areas marked by squares in A and D respectively. C show focal positive expression of α -SMA in mLSCC. F show stromal negative expression of α -SMA in SIL (This case is high-grade SIL).

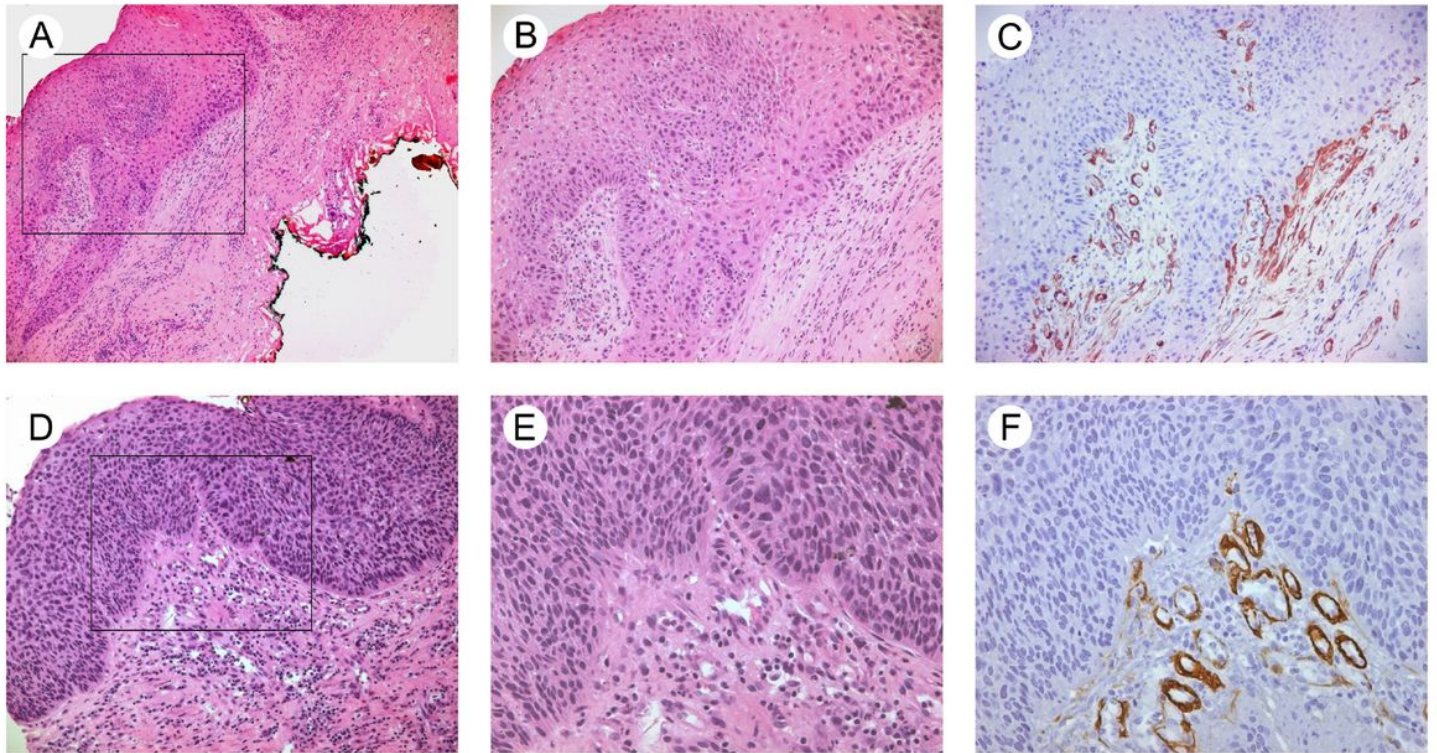


Figure 1

Comparison of stromal α -SMA expression in mLSCC (A-C) and laryngeal SIL (D-F). B and E show the areas marked by squares in A and D respectively. C show focal positive expression of α -SMA in mLSCC. F show stromal negative expression of α -SMA in SIL (This case is high-grade SIL).

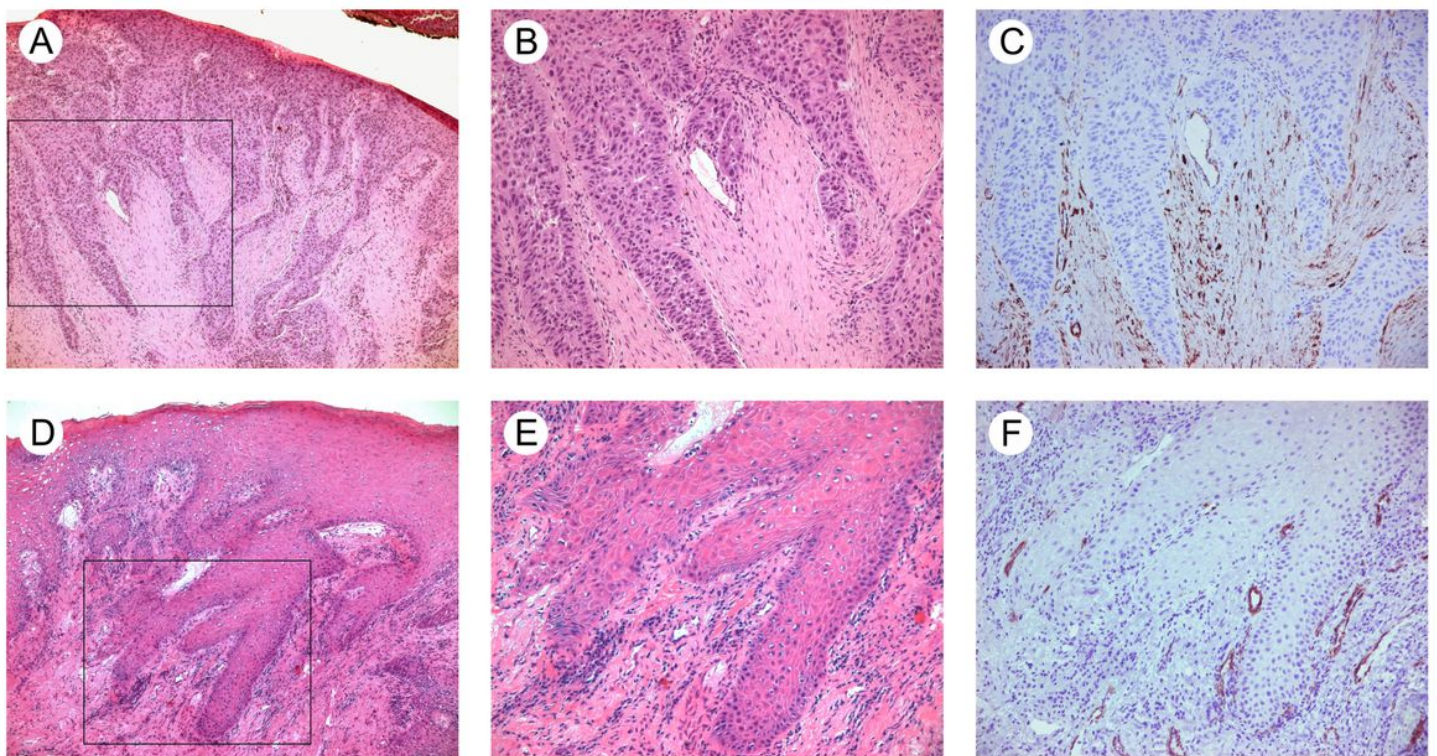


Figure 2

Comparison of stromal α -SMA expression in mLSCC (A-C) and laryngeal PEH (D-F). B and E show the areas marked by squares in A and D respectively. C show diffuse positive expression of α -SMA in mLSCC. F show stromal negative expression of α -SMA in PEH.

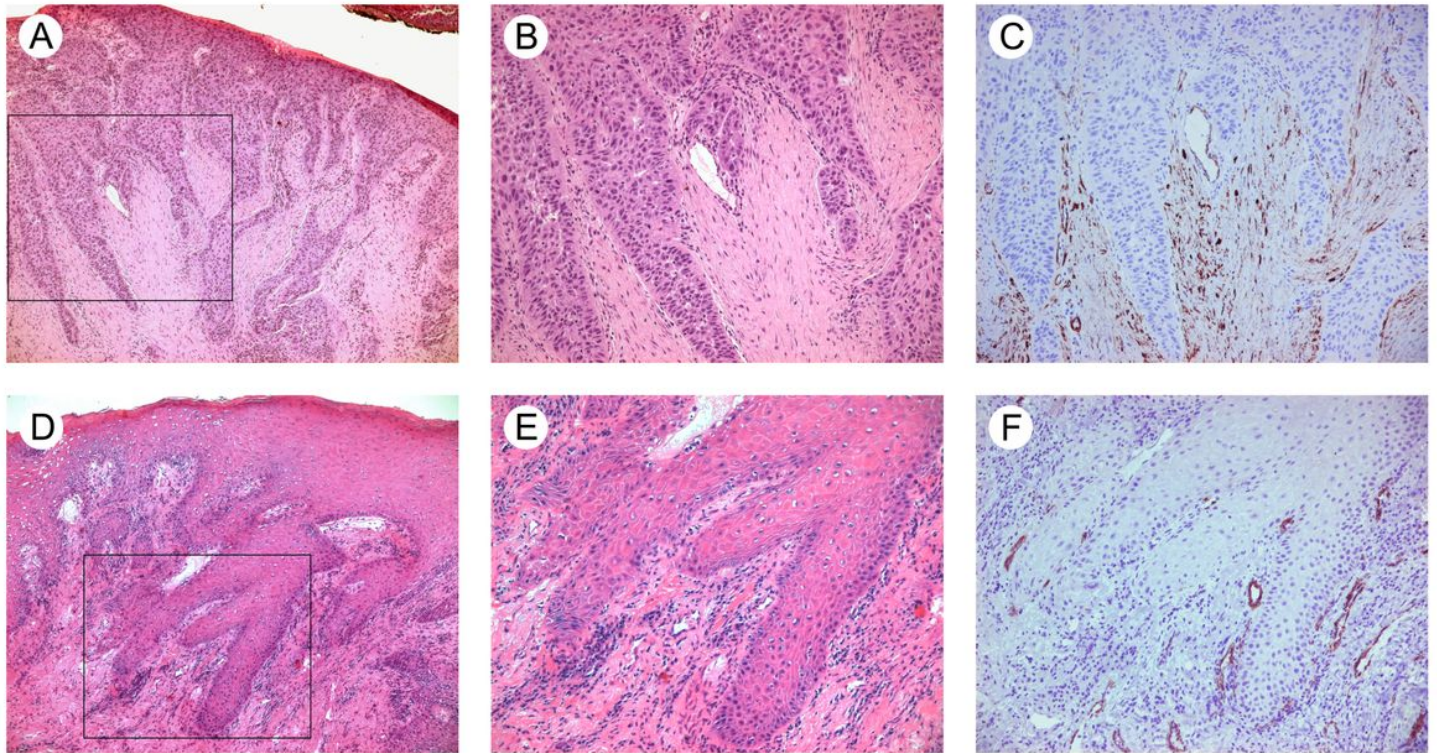


Figure 2

Comparison of stromal α -SMA expression in mLSCC (A-C) and laryngeal PEH (D-F). B and E show the areas marked by squares in A and D respectively. C show diffuse positive expression of α -SMA in mLSCC. F show stromal negative expression of α -SMA in PEH.

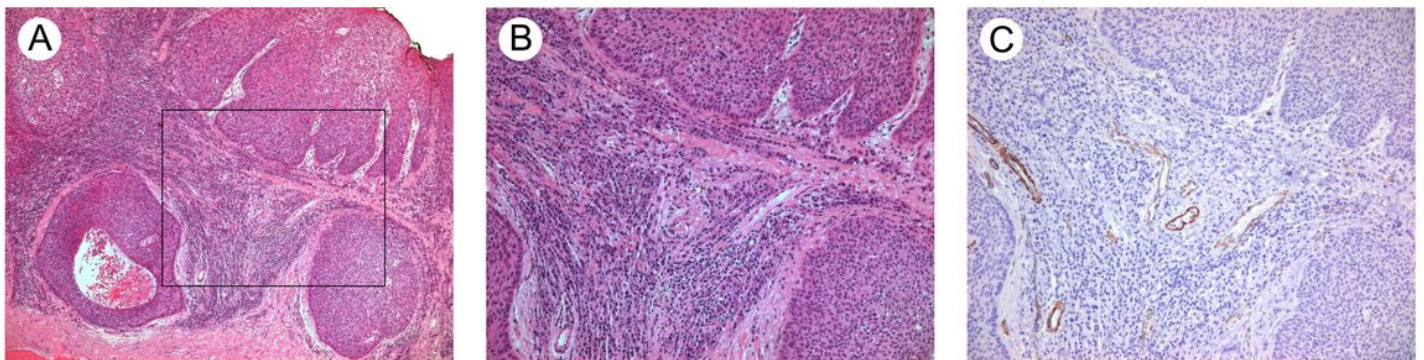


Figure 3

MLSCC with dense lymphoplasmacytic infiltration in the stroma. A show an expansive growth pattern at the invasive front of the tumor and dense lymphoplasmacytic infiltration in the stroma. B show the area marked by the square in A. C show stromal negative expression of α -SMA.

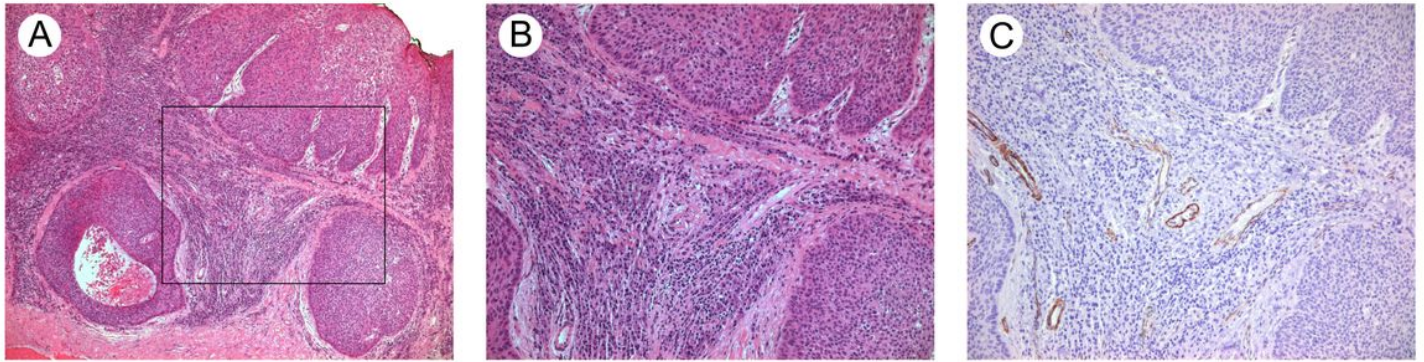


Figure 3

MLSCC with dense lymphoplasmacytic infiltration in the stroma. A show an expansive growth pattern at the invasive front of the tumor and dense lymphoplasmacytic infiltration in the stroma. B show the area marked by the square in A. C show stromal negative expression of α -SMA.