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Significance of myofibroblast appearance in squamous cell carcinoma of the oral cavity on the occurrence of occult regional metastases, distant metastases, and survival

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Running title: Myofibroblast appearance in oral cancer

ABSTRACT

The aim of the present study was to assess the frequency of stromal myofibroblasts appearance, and to further clarify whether myofibroblasts influence tumor suppression or progression. Surgical resection specimens from 152 patients with cT1-T3N0 oral squamous cell carcinoma (OSCC) were analysed. Frequency of myofibroblasts within tumoral stroma was assessed immunohistochemically and compared with other clinically and histopathological factors. Immunohistochemical reaction for alpha-smooth muscle actin showed positive cells in stroma of 84.2% (n=128) of OSCC. Increased presence of myofibroblasts in the tumor stroma was significantly correlated with T stage ($P = 0.019$), presence of occult neck metastases ($P < 0.001$), regional recurrence ($P = 0.037$), and distant metastases ($P = 0.008$). There was also association between presence of myofibroblasts and patient's survival ($P = 0.009$). Presence of myofibroblasts was not associated with local recurrence, tumor cellular differentation, mode of invasion, and bone invasion. Results of this study suggest that myofibroblast proliferation facilitates tumour invasion, occurrence of occult neck disease and distant metastases. Survival rate was poorer in patients with abundant myofibroblasts. Further investigations on tumor-associated stroma at invasive front are needed in order to provide new diagnostic markers and therapeutic strategies.

Keywords: head and neck; oral cancer; myofibroblast; prognostic factor

INTRODUCTION

Oral cancer is common worldwide, particularly in developing countries, where it constitutes up to 25% of all malignancies[1]. Squamous cell carcinoma is the most common histological type of oral cancer. It is associated with great morbidity and mortality rates that have not improved in decades despite early detection and therapeutic advances[2]. Extensive research in the field of tumorigenesis has shown that tumor progression depends on its genetic characteristics and on interactions with its environment[3]. The tumor microenvironment comprise cancer cells, stromal cells and extracellular matrix (ECM)[4]. One of the major components of tumor-associated stroma are fibroblasts and myofibroblasts. Due to their ability to produce collagen and ECM proteins, it has been suggested that these cells represent an important factor in the development of the desmoplastic reaction that facilitates invasion both in vitro and in vivo[5-7]. Most of the biological markers of metastases are derived from cancer cells rather than the stroma. However, assessment of the tumor-host borderline provides a correlation with prognosis and understanding the mode of tumour cell invasion which enables prediction of the treatment outcome[8-10]. The aim of the present study is to assess the abundance of stromal myofibroblasts (SMF), and to correlate it with cancer recurrence and patient's survival. In addition, we investigated the relationship between myofibroblast appearance and the incidence of occult neck disease in order to clarify the indications for elective lymphadenectomy and the outcome of treatment.

MATERIALS AND METHODS

Informed consent was obtained from each patient and the study was carried out with the approval of the Ethical Committee of the University of Zagreb School of Medicine, University Hospital Dubrava. The study included 152 consecutive patients with cT1-T3N0 oral squamous cell carcinoma (OSCC) who were diagnosed and surgically treated between 2000 and 2004 at the Department of Maxillofacial Surgery, University Hospital Dubrava, Zagreb, Croatia. Inclusion criteria were: primary surgical treatment, no clinical evidence of regional metastases (cN0), no prior treatment for OSCC, no significant comorbidities, and a follow up period of at least six months. Standard surgical treatment included intraoral excision for cT1 tumors and intraoral excision +/- elective neck dissection for cT2-T3 tumors. Bilateral lymphadenectomy was performed only in cases when primary tumors approached the midline. Patient monitoring was concluded on the 31st of October 2007.

Staging of the disease was based on the international TNM classification from 2009.[11] Patients with adverse histopathological features (positive margin, perineural invasion, extracapsular spread, pT3 or pT4 primary) underwent postoperative irradiation. With daily fractions of 2 Gy, a prophylactic dose of 50 Gy to uninvolved neck levels was given, followed by 60 Gy to the tumor bed with a boost of 66 Gy being applied to sites of increased recurrence risk, especially regions of the neck with extracapsular nodal disease and microscopically involved margins. Tumor volumes, (mm^3), were calculated from measurements of the tumour diameter and depth of invasion. It was assumed that tumors are approximately cones whose volume is $r^2\pi h/3$. Margin status was defined as negative when the tumor was ≥4mm from the inked surgical margin. Immunohistochemistry was performed by sensitive peroxidase-streptavidin method on formalin-fixed, paraffin embedded tissue. Four μm-thick sections were cut from 152 blocks containing representative specimen of study

cases and stained with monoclonal antibody against alpha-smooth muscle actin (α-SMA) diluted 1:200 (Dako, Glostrup, Denmark). Proliferation of myofibroblasts within tumour stroma was assesed semiquantitatively based on a 4-scale scoring system: 0 = no myofibroblasts, 1 = present up to 25% of stroma, 2 = present in 26-50% of stroma, 3 = present in 51-75% of stroma, and 4 = present in more than 76% of stroma. For purposes of statistical analysis, cases with low scores (0 and 1) were combined and compared to cases with high scores (2, 3 and 4).

The mode of cancer invasion was evaluated according to the classification proposed by Yamammoto[8], and modified by Bryne et al.[12] where only the most invasive parts of the tumor (deep invasive margins) were graded.

Follow-up protocol consisted of history and physical examination every 3, 6, 9 and 12 months, in the first, second, third, and fourth year of follow-up, respectively. Post-treatment CT scans (oral cavity and neck) were performed within 1 and 5 years after surgical treatment. Other repeated imaging was done only in patients with signs/symptoms, not routinely in asymptomatic patients.

Statistical Analysis

Numerical data were presented with mean and standard deviation and categorical with absolute and relative frequencies. Follow-up intervals were calculated in months from the date of first treatment to the date of last follow-up or death. Association between presence of myofibroblasts and clinicopathological parameters was performed by Chi-square test or Fisher's exact test when appropriate. Multivariate analysis of variables related to regional metastases was done using logistic regression. The Kaplan-Meier method was applied for survival analysis, and the statistical significance was evaluated using log-rank test. Regression data were presented with regression coefficients and odds ratio with 95% confidence intervals

(95% CIs). All statistics was done using MedCalc statistical software (MedCalc Statistical Software ver. 13.0.2, MedCalc Software bvba, Ostend, Belgium). P values of < 0.05 were considered statistically significant.

RESULTS

Over the 5-year period, 183 patients were primarily surgically treated. After eliminating patients who did not meet inclusion criteria, 152 patients were egliable for study. The study group included 124 men (81.6%) and 28 (18.4%) women with a median age of 59 (range 34-85 years). Clinicopathological characteristics of the study group are summarized in **Table 1**. Immunohistochemical reaction for α-SMA showed positive cells in the stroma of 84.2% (n = 128) of OSCC. Scanty presence of myofibroblasts was observed in 42 (32.8%) of OSCC positive samples, whereas 86 (67.2%) samples demonstrated an abundant distribution of myofiboblasts (Figure 1, 2 and 3). The univariate clinicopathological correlations with the presence of myofibroblasts are listed in Table 2. Abundant presence of myofibroblasts in the tumor stroma significantly correlated with T stage, presence of occult neck metastases, regional recurrence, and distant metastases. There was also an association between the presence of myofibroblasts and patient's survival. Patients whose specimens demonstrated abundant myofibroblasts had a disease specific survival rate of 67.2% at 5-years compared with 91.9% for patients with scanty myofibroblasts which is statistically significant (Figure 4). Presence of myofibroblasts was not associated with age, gender, local recurrence, tumor cell differentation, mode of invasion, bone invasion, and margin status. We also carried out a multivariate analysis, which identified high myofibroblast scores and poorly differantiated tumors as unfavorable prognostic factors with respect to occult neck metastases (Table 3). The odds of occult neck disease for patients with high myofibroblast scores and poorly differentiated tumors were 8.68 and 2.31 higher compared to patients with low myofibroblasts scores and moderate/well differantiated tumors, respectively ($P < 0.001$, 95% CI: 2.87-26.29; $P = 0.044$, 95% CI: 1.02-5.25). Postoperative radiotherapy was administered to 69 of 152 (45.4%) patients. At the end of the study, thirty-four of the patients had died (22.4%) of

disease recurrence. Follow-up information was available for all patients and ranged from 7 to 91 months (mean 43).

DISCUSSION

The most significant prognostic factor of survival for patients with OSCC is the status of regional lymph nodes. The presence of regional metastases reduces survival of these patients up to 50% and indicates the higher risk for regional recurrence and distant metastasis[13,14]. However, indications for elective neck dissection in early OSCC are still controversial. The aim of the present study was to analyse the relationship between appearance of SMF and the incidence of occult neck disease, distant metastases and survival of patients with cT1-T3N0 OSCC in order to clarify the indications for elective neck dissection. To our knowledge this is the largest study in the English literature examining the role of SMF proliferation in squamous cell carcinoma of the oral cavity and the first one analysing the role of its proliferation in the context of the occurrence of occult neck disease and distant metastases. SMF appearance has recently been shown to be associated with significantly lower survival rates in several types of carcinomas including breast and colorectal[15,16]. However, there are only few studies in the English literature regarding the role of these cells in OSCC. Lewis et al[5] studied in vitro cell lines of OSCC and observed that cancer cells through transforming growth factor beta (TGF-β) secretion induce myofibroblast differentiation, while myofibroblasts promoted cancer cell invasion into matrix gell through high hepatocyte growth factor (HGF) secretion. These findings imply the existence of a double paracrine mechanism between cancer cells and myofibroblasts. Similar results were obtained by Kellerman et al[17].

Sobral et al[18] showed that myofibroblast clones induce increased production of metalloproteinases by cancer cells. Additionally, the authors demonstrated that myofibroblasts promote the proliferative activity of OSCC by upregulating activin A, a member of the TGF-β superfamily of proteins. In an experimental study on carcinogenesis with 4NQO in the tongue mucosa of Wistar rats, Vered et al[19] showed that SMF were not

associated with normal, hyperkeratotic/hyperplastic and dysplastic epithelium, irrespective of location along the tongue and the severity of the dysplastic changes, suggesting that increased number of SMF occurred simultaneously with the appearance of the carcinoma. In agreement with previous findings, Kellerman et al[20], confirmed the presence of myofibroblasts only in the tumour stroma, especially at the invasive front of the carcinoma. This is completely opposite to what has been reported in other types of human carcinoma in which myofibroblasts were found prior to the invasive process[21-23]. The findings in our study, which showed a statistically significant association between the frequency of myofibroblast appearance and poorer survival rates, are in accordance with prior reports[20,24-26]. On the other hand, some authors did not find a significant association between myofibroblasts and patients survival[17]. SMF scores were statistically insignificant with respect to local recurrence (P = 0.064). On the contrary, Vered et al[24] observed that abundance of SMF had an independent adverse effect on local control. In our study, lymph node metastases and regional recurrence occurred more frequently in the abundant myofibroblast group, which was also demonstrated by the findings of other authors[17,26]. However, this is the first study addressing its proliferation regarding occurrence of occult neck disease. Furthermore, to our knowledge, this is the first evidence showing a significantly increased occurrence of distant metastases in patients with abundant myofibroblast appearance. Myofibroblast appearance increased with increasing tumor invasiveness, although no statistically significant correlation was found (P = 0.089). In a series of 84 biopsy materials from patients with OSCC, Kawashiri et al[26] observed similar results, suggesting that the frequency of these cells increased gradually with mode of invasion. In addition, de-Assis et al[27] found that the presence of SMF was higher in tumors with a more diffuse histological pattern of invasion.

Recent evidence suggests that myofibroblasts represent the main effector of tumor-associated stroma, which facilitate the invasion process and inhibit the host immune response. Also there is increasing evidence that some of SMF actually represent epithelial malignant cells that have undergone epithelial-mesenhymal transition (EMT), the process by which tumour cells lose E-cadherin-dependent intercellular adhesion and enhance motility, thus becoming able to penetrate into surrounding tissues[28]. This hypothesis was further supported by the triple immunostaining procedure, which showed that loss of E-cadherin among carcinoma cells being in direct contact with adjacent SMF[24]. Furthermore, carcinoma cells can induce a reversal of these changes, termed mesenchymal-to-epithelial transitions (METs), by which carcinoma-derived stromal cells adopt epithelial characteristics establishing new tumour foci, which could be a reasonable explanation for the high disease reccurence and poorer survival rates obtained when an abundance of myofibroblasts was found[29].

In addition, some authors demonstrated that interaction of SMF with OSCC cells leads to an activation of the EGFR signalling system in tumor with critical role not only in increased proliferation and development of EMT but also in intratumor lymphangiogenesis[30]. These results indicate possible influence of EGFR-inhibitor therapy.

Finally, administration of a fibroblast inhibitor (transilat) used to treat hypertrophic scars and keloids in an *in vivo* model significantly suppresed tumor growth and cervical lymph node metastasis occurence in nude mice[26]. This biological interaction may be clinically relevant targeted therapy in patients with oral squamous cell carcinoma.

This study has all limitations associated with retrospective study. Another clear weakness of this analysis is inability to test molecular mechanisms by which myofibroblasts contribute to

biological aggressivness of OSCCs. Although we have demonstrated that increase in myofibroblasts correlates with higher T stage, another possible explanation for this phenomenon is that myofibroblasts could just be a marker for more advanced disease and as such have no prognostic value beyond the clinical T size. Further investigations are needed to clarify whether these stromal cells can act as separate prognostic marker, or whether they merely reflect primary tumor size. Also, large prospective studies are necessary to clarify if the number of myofibroblasts in the tumor stroma (determined preoperatively in biopsy material) can help in decision making regarding the need for elective neck dissection or radiotherapy after surgical treatment. On the other hand, we believe that this data provide insights into important prognosticators which may be useful to define optimal treatment of OSCC in elective settings.

In conclusion, we have demonstrated that an abundant presence of myofibroblasts leads to a more aggressive phenotype of the OSCCs, resulting in a significantly increased presence of occult metastases, regional recurrence and distant metastases as well as lower survival rates. The multivariate model derived from this study identified high myofibroblast score as independent predictor of occult neck disease thus determining the patients most likely to benefit from elective neck dissection. Therefore, myofibroblasts in oral cancer facilitate tumor growth and metastatic spread. Further investigations of stromal interactions and phenotypic characteristics of myofibroblasts at the invasive front are needed in order to provide new diagnostic markers or anti-cancer therapeutic modalities.

Declarations

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Competing Interests: None declared.

Ethical Approval: This study has been approved by the Ethical Committee of the University Hospital Dubrava in which it was performed.

Patient Consent: Not required

References

1. Pisani P, Bray F, Parkin DM. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. Int J Cancer 2002: 97: 72-81.

2. Lippman SM, Sudbø J, Hong WK. Oral cancer prevention and the evolution of molecular-targeted drug development. J Clin Oncol 2005: 23: 346-356.

3. Galiè M, Sorrentino C, Montani M, Micossi L, Di Carlo E, D'Antuono T, Calderan L, Marzola P, Benati D, Merigo F, Orlando F, Smorlesi A, Marchini C, Amici A, Sbarbati A. Mammary carcinoma provides highly tumourigenic and invasive reactive stromal cells. Carcinogenesis 2005: 26 : 1868-1878.

4. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature 2001: 411: 375-379.

5. Lewis MP, Lygoe KA, Nystrom ML, Anderson WP, Speight PM, Marshall JF, Thomas GJ. Tumour-derived TGF-beta1 modulates myofibroblast differentiation and promotes HGF/SF-dependent invasion of squamous carcinoma cells. Br J Cancer 2004: 90: 822-832.

6. Barth PJ, Schenck zu Schweinsberg T, Ramaswamy A, Moll R. CD34+ fibrocytes, alpha-smooth muscle antigen-positive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx, and larynx. Virchows Arch 2004: 444: 231-234.

7. Vered M, Shohat I, Buchner A, Dayan D. Myofibroblasts in stroma of odontogenic cysts and tumors can contribute to variations in the biological behavior of lesions. Oral Oncol 2005: 41: 1028-1033.

8. Yamamoto E, Kohama G, Sunakawa H, Iwai M, Hiratsuka H. Mode of invasion, bleomycin sensitivity, and clinical course in squamous cell carcinoma of the oral cavity. Cancer 1983: 51: 2175-2180.

9. Sasaki T, Moles DR, Imai Y, Speight PM. Clinico-pathological features of squamous cell carcinoma of the oral cavity in patients <40 years of age. J Oral Pathol Med 2005: 34: 129-133.

10. Miyamoto R, Uzawa N, Nagaoka S, Nakakuki K, Hirata Y, Amagasa T. Potential marker of oral squamous cell carcinoma aggressiveness detected by fluorescence in situ hybridization in fine-needle aspiration biopsies. Cancer 2002: 95: 2152-2159.

11. Sobin LH, Gospodarowicz MK, Wittekind Ch, editors. TNM Classification of Malignant Tumors, 7th Edition. International Union Against Cancer. Hoboken, NJ: Wiley-Blackwell, 2010.

12. Bryne M1, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol 1992: 166 : 375-381.

13. Leemans CR, Tiwari R, Nauta JJ, van der Waal I, Snow GB. Regional lymph node involvement and its significance in the development of distant metastases in head and neck carcinoma. Cancer. 1993: 71: 452-456.

14. Shah JP, Lydiatt W. Treatment of cancer of the head and neck. CA Cancer J Clin 1995: 45: 352-368.

15. Surowiak P, Murawa D, Materna V, Maciejczyk A, Pudelko M, Ciesla S, Breborowicz J, Murawa P, Zabel M, Dietel M, Lage H. Occurence of stromal myofibroblasts in the invasive ductal breast cancer tissue is an unfavourable prognostic factor. Anticancer Res 2007 :27: 2917-2924.

16. Tsujino T, Seshimo I, Yamamoto H, Ngan CY, Ezumi K, Takemasa I, Ikeda M, Sekimoto M, Matsuura N, Monden M. Stromal myofibroblasts predict disease recurrence for colorectal cancer. Clin Cancer Res. 2007: 13: 2082-2090.

17. Kellermann MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, Kowalski LP, Coletta RD. Mutual paracrine effects of oral squamous cell carcinoma cells and normal oral fibroblasts: induction of fibroblast to myofibroblast transdifferentiation and modulation of tumor cell proliferation. Oral Oncol 2008: 44: 509-517.

18. Sobral LM, Bufalino A, Lopes MA, Graner E, Salo T, Coletta RD. Myofibroblasts in the stroma of oral cancer promote tumorigenesis via secretion of activin A. Oral Oncol 2011: 47: 840-846.

19. Vered M, Allon I, Buchner A, Dayan D. Stromal myofibroblasts and malignant transformation in a 4NQO rat tongue carcinogenesis model. Oral Oncol 2007: 43: 999-1006.

20. Kellermann MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, Nishimoto I, Kowalski LP, Coletta RD. Myofibroblasts in the stroma of oral squamous cell carcinoma are associated with poor prognosis. Histopathology 2007: 51: 849-853.

21. Sappino AP, Skalli O, Jackson B, Schürch W, Gabbiani G. Smooth-muscle differentiation in stromal cells of malignant and non-malignant breast tissues. Int J Cancer 1988: 41: 707-712.

22. Cintorino M, Bellizzi de Marco E, Leoncini P, Tripodi SA, Xu LJ, Sappino AP, Schmitt-Gräff A, Gabbiani G. Expression of alpha-smooth-muscle actin in stromal cells of the uterine cervix during epithelial neoplastic changes. Int J Cancer 1991: 47: 843-846.

23. Martin M, Pujuguet P, Martin F. Role of stromal myofibroblasts infiltrating colon cancer in tumor invasion. Pathol Res Pract 1996: 192: 712-717.

24. Vered M, Dobriyan A, Dayan D, Yahalom R, Talmi YP, Bedrin L, Barshack I, Taicher S. Tumor-host histopathologic variables, stromal myofibroblasts and risk score, are significantly associated with recurrent disease in tongue cancer. Cancer Sci 2010: 101: 274-280.

25. Bello IO, Vered M, Dayan D, Dobriyan A, Yahalom R, Alanen K, Nieminen P, Kantola S, Läärä E, Salo T. Cancer-associated fibroblasts, a parameter of the tumor microenvironment,

overcomes carcinoma-associated parameters in the prognosis of patients with mobile tongue cancer. Oral Oncol 2011: 47: 33-38.

26. Kawashiri S, Tanaka A, Noguchi N, Hase T, Nakaya H, Ohara T, Kato K, Yamamoto E. Significance of stromal desmoplasia and myofibroblast appearance at the invasive front in squamous cell carcinoma of the oral cavity. Head Neck 2009: 31: 1346-1353.

27. de-Assis EM, Pimenta LG, Costa-e-Silva E, Souza PE, Horta MC. Stromal myofibroblasts in oral leukoplakia and oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2012: 17: 733-738.

28. Guarino M. Epithelial-mesenchymal transition and tumour invasion. Int J Biochem Cell Biol. 2007: 39: 2153-2160.

29. Hugo H, Ackland ML, Blick T, Lawrence MG, Clements JA, Williams ED, Thompson EW. Epithelial-mesenchymal and mesenchymal-epithelial transitions in carcinoma progression. J Cell Physiol 2007: 213: 374-383.

30. Berndt A, Büttner R, Gühne S, Gleinig A, Richter P, Chen Y, et al. Effects of activated fibroblasts on phenotype modulation, EGFR signalling and cell cycle regulation in OSCC cells. Exp Cell Res 2014: 322: 402-414.

CAPTIONS TO FIGURES

Figure 1. Less than 25% of tumor-associated stromal cells express SMA reactivity

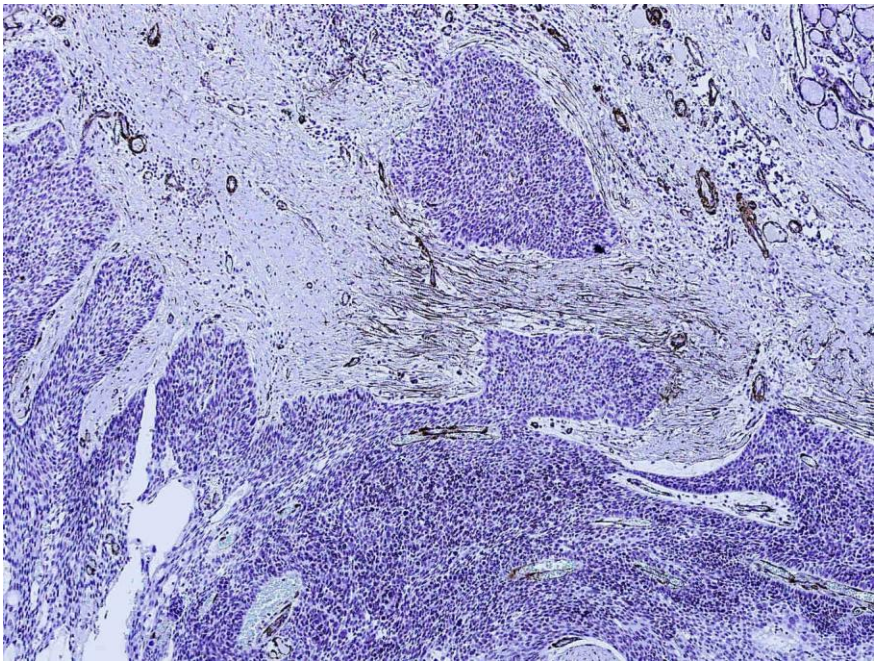

Figure 2. Approximately 50% of tumor-associated stromal cells express SMA reactivity

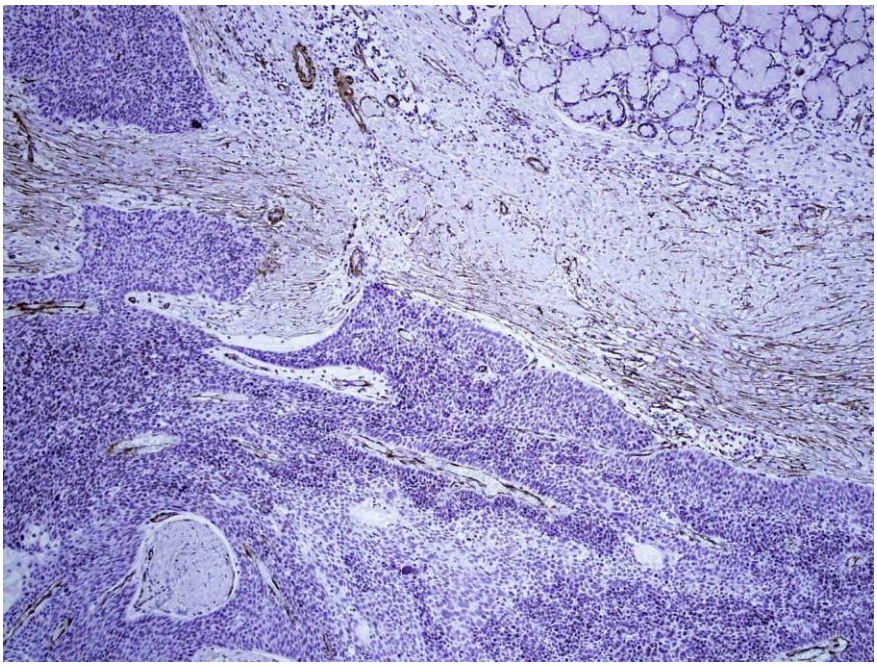

Figure 3. Almost 100% of tumor-associated stromal cells express SMA reactivity

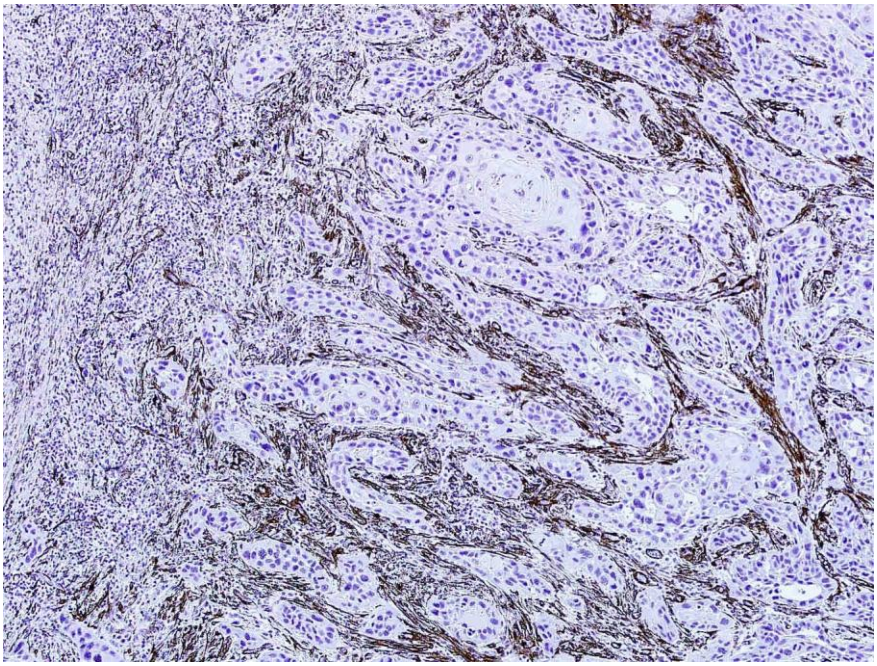

Figure 4. Disease-specific survival of the patients with abundant myofibroblasts and patients with scanty myofibroblast appearance. The two curves are statistically significant different (P = 0.009).

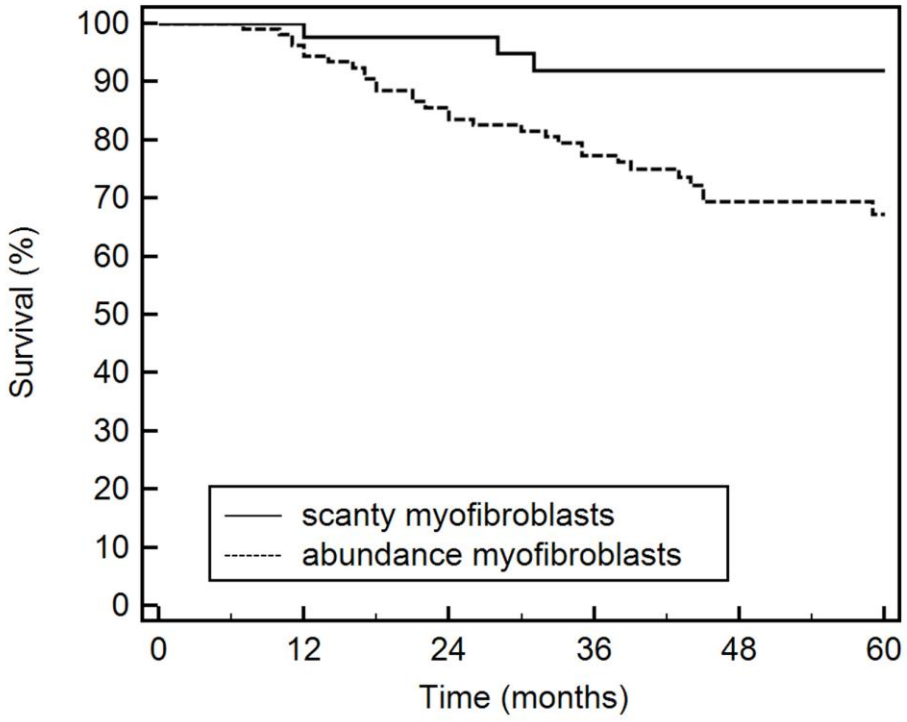

Table 1. Clinicopathological features of 152 patients

Characteristics	Number of patients (%)
Age (years)	
≤60	85 (55.9)
>60	67 (44.1)
Gender	
Male	124 (81.6)
Female	28 (18.4)
Primary site	
Flour of the mouth	72 (47.3)
Tongue	43 (28.3)
Retromolar space	22 (14.5)
Lower gingiva	15 (9.9)
T classification	
T1	37 (24.3)
T2	98 (64.5)
T3	17 (11.2)
Margin status	
Negative	142 (93.4)
Positive	10 (6.6)
Differentiation	
Well	72 (47.4)
Moderate	43 (28.3)
Poor	37 (24.3)
Mode of invasion	
1	10 (6.6)
2	28 (18.4)
3	53 (34.9)
4	61 (40.1)
Perineural invasion	
No	74 (48.7)
Yes	78 (51.3)
Recurrence	
Local	20 (13.1)
Regional	12 (7.9)
Distant	21 (13.8)
Survival	
Alive	118 (77.6)
Dead	34 (22.4)

Table 2. Correlation of the myofibroblasts appearance and clinocopathological data.

Parameters	Myofibroblasts		P-value
	low scores	high scores	
Age			
≤ 60	21	64	
> 60	21	46	0.368
Gender			
Male	34	90	
Female	8	20	1.00
T stage			
T1	15	22	
T2	25	73	
T3	2	15	0.019
N stage			
pN0	38	58	
pN+	4	52	<0.001
Local recurrence			
No	40	92	
Yes	2	18	0.064
Regional recurrence			
No	42	98	
Yes	0	12	0.037
Distant metastases			
No	41	90	
Yes	1	20	0.008
Mode of invasion			
1	4	6	
2	11	17	
3	13	40	0.089
4	14	47	
Bone invasion			
No	15	46	
Yes	4	19	0.570[a]
Margin			
Negative	39	103	
Positive	3	7	1.00

Table 3. Univariate and multivariate analysis of variables related to regional metastases.

Parameter	Regional metastases		Univariate statistics			Multivariate statistics*
	No	Yes	Regression coefficients	Probability	Odds ratio	Odds ratio
	N (%)	N (%)	b ± SE (b)	P value	OR (95% CI)	OR (95% CI)
Age						
≤ 60	51 (33.5)	34 (22.4)	–			
> 60	45 (29.6)	22 (14.5)	–0.31 ± 0.34	0.364	0.73 (0.38-1.43)	–
Gender						
Male	78 (51.3)	46 (30.3)	–			
Female	18 (11.8)	10 (6.6)	–0.06 ± 0.44	0.891	0.94 (0.4-2.21)	–
T stage						
T1	26 (17.1)	11 (7.2)	–			
T2	61 (40.1)	37 (24.4)	0.36 ± 0.42	0.386	1.43 (0.64-3.24)	–
T3	9 (5.9)	8 (5.3)	0.74 ± 0.6	0.219	2.1 (0.64-6.87)	
Tumor thickness						
≤ 5mm						
> 5mm						
Tumor volume	63 (65.6)	33 (58.9)	0.29 ± 0.35	0.409	1.33 (0.67-2.62)	
≤ 1cm^3	33 (34.4)	23 (41.1)				
> 1cm^3						
Primary site						
Flour of the mouth	45 (29.6)	27 (17.8)	–			
Tongue	25 (16.4)	18 (11.8)	0.18 ± 0.39	0.643	1.2 (0.56-2.59)	_

Retromolar space	14 (9.2)	8 (5.3)	–0.05 ± 0.5	0.923	0.95 (0.35-2.57)	
Lower gingiva	12 (7.9)	3 (2.0)	–0.88 ± 0.69	0.204	0.42 (0.11-1.61)	
Margin status						
Negative	89 (58.5)	53 (34.9)	–			
Positive	7 (4.6)	3 (2.0)	–0.33 ± 0.71	0.644	0.72 (0.18-2.9)	–
Differentiation	52 (34.2)	20 (13.2)	–			
Well	26 (17.1)	17 (11.2)	0.53 ± 0.41	0.194	1.7 (0.76-3.78)	1.5 (0.64-3.54)
Moderate	18 (11.8)	19 (12.5)	1 ± 0.42	0.017	2.74 (1.2-6.27)	2.31 (1.02-5.25)
Poor						
Perineural invasion						
No	53 (34.9)	21 (13.8)	–			
Yes	43 (28.3)	35 (23.0)	0.72 ± 0.34	0.036	2.05 (1.04-4.03)	1.44 (0.69-2.99)
Myofibroblasts						
low scores	38 (25)	4 (2.6)				
high scores	58 (38.2)	52 (34.2)	2.14 ± 0.56	<0.001	8.52 (2.85-25.49)	8.68 (2.86-26.29)
Laminin						
Fibronectin						
CD 34						

*Only factors which demonstrated statistical significancy on univariate analysis were submitted to multivariate statistics