Int. J. Oral Maxillofac. Surg. 2015; 44: 1075–1080
http://dx.doi.org/10.1016/j.ijom.2015.05.009, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Head and Neck Oncology

Clinical Paper

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

I. Luksic[1], P. Suton[2], S. Manojlovic[3], M. Virag[1], M. Petrovecki[4], D. Macan[5]

[1]Department of Maxillofacial Surgery, University of Zagreb School of Medicine, University Hospital Dubrava, Zagreb, Croatia; [2]Department of Radiation Oncology, University Hospital for Tumours, University Hospital Centre Sisters of Mercy, Zagreb, Croatia; [3]Department of Pathology and Cytology, University of Zagreb School of Medicine, University Hospital Dubrava, Zagreb, Croatia; [4]Department of Medical Informatics, University of Rijeka School of Medicine, Rijeka, Croatia; [5]Department of Maxillofacial Surgery, Division of Oral Surgery, University of Zagreb School of Dental Medicine, University Hospital Dubrava, Zagreb, Croatia

I. Luksic, P. Suton, S. Manojlovic, M. Virag, M. Petrovecki, D. Macan: Significance of myofibroblast appearance in squamous cell carcinoma of the oral cavity on the occurrence of occult regional metastases, distant metastases, and survival. Int. J. Oral Maxillofac. Surg. 2015; 44: 1075–1080.

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

Key words: head and neck; oral cancer; myofibroblast; prognostic factor.

Accepted for publication 14 May 2015
Available online 6 June 2015

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 high morbidity and mortality rates, which have not improved in decades despite early detection and therapeutic advances.[2] Extensive research in the field of tumourigenesis has shown that tumour progression depends on its genetic characteristics and on interactions with its environment.[3]

The tumour microenvironment comprises cancer cells, stromal cells, and extracellular matrix.[4] Fibroblasts and myofibroblasts form a major component of the tumour-associated stroma. Due to their ability to produce collagen and extracellular matrix 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 tumour–host interface provides a correlation with the prognosis and an understanding of the mode of tumour cell invasion, enabling the treatment outcome to be predicted.[8–10] The aim of the present study was to assess the abundance of stromal myofibroblasts in squamous cell carcinoma of the oral cavity, and to correlate this with cancer recurrence and patient survival. In addition, the relationship between myofibroblast appearance and the incidence of occult neck disease was investigated 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 ethics committee of the study institution. The study included 152 consecutive patients with cT1–T3N0 oral squamous cell carcinoma (OSCC) who were diagnosed and treated surgically between 2000 and 2004 in the department of maxillofacial surgery of the university hospital in Zagreb, Croatia. Inclusion criteria were the following: primary surgical treatment, no clinical evidence of regional metastasis (cN0), no prior treatment for OSCC, no significant comorbidities, and a follow-up period of at least 6 months.

Standard surgical treatment included intraoral excision for cT1 tumours and intraoral excision with or without elective neck dissection for cT2–T3 tumours. A bilateral lymphadenectomy was performed only in cases where the primary tumours approached the midline. Patient monitoring was concluded on 31 October 2007.

Staging of the disease was based on the international TNM classification of 2009.[11] Patients with adverse histopathological features (positive margin, perineural invasion, extracapsular spread, pT3 or pT4 primary) underwent postoperative irradiation. In daily fractions of 2 Gy, a prophylactic dose of 50 Gy was given to uninvolved neck levels, followed by 60 Gy to the tumour 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. Tumour volumes (mm^3) were calculated from measurements of the tumour diameter and depth of invasion. It was assumed that tumours are approximately cones with a volume of $\pi r^2 h/3$.

Margin status was defined as negative when the tumour was $\geq$4 mm from the inked surgical margin. Immunohistochemistry was performed by sensitive peroxidase–streptavidin method on formalin-fixed, paraffin-embedded tissue. Sections 4 μm in thickness were cut from 152 blocks containing representative specimens of the study cases and stained with monoclonal antibody against alpha-smooth muscle actin (α-SMA) diluted 1:200 (Dako, Glostrup, Denmark). The proliferation of myofibroblasts within the tumour stroma was assessed semi-quantitatively with a 5-point scoring system: 0 = no myofibroblasts, 1 = present in up to 25% of the stroma, 2 = present in 26–50% of the stroma, 3 = present in 51–75% of the stroma, and 4 = present in more than 76% of the stroma. For the 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 Yamamoto et al.,[8] and modified by Bryne et al.[12]; only the most invasive parts of the tumour (deep invasive margins) were graded.

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

Statistical analysis

Numerical data were recorded as the mean and standard deviation; categorical data were recorded as the 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. The association between the presence of myofibroblasts and clinicopathological parameters was assessed with the χ^2 test or Fisher's exact test, as appropriate. Multivariate analysis of variables related to regional metastasis was done using logistic regression. The Kaplan–Meier method was applied for survival analysis, and the statistical significance was evaluated using the log-rank test. Regression data were reported as regression coefficients and the odds ratio (OR) with 95% confidence interval (95% CI). All statistical analyses were done using MedCalc statistical software version 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 underwent primary surgical treatment. After eliminating patients who did not meet the inclusion criteria, 152 were eligible for the present study. The study group included 124 men (81.6%) and 28 (18.4%) women, with a median age of 59 years (range 34–85 years). Clinicopathological characteristics of the study group are summarized in Table 1.

The immunohistochemical reaction for α-SMA showed positive cells in the stroma of 84.2% ($n = 128$) of OSCC. Myofibroblasts were scarce in 18 (14.1%) of the OSCC positive samples, whereas 110 (85.9%) samples demonstrated an abundant distribution of myofibroblasts (Figs 1–3).

The univariate correlation of myofibroblast distribution with clinicopathological data is given in Table 2. An abundant presence of myofibroblasts in the tumour stroma was found to be significantly correlated with T stage ($P = 0.019$), the presence of occult neck metastasis ($P < 0.001$), regional recurrence ($P = 0.037$), and distant metastasis ($P = 0.008$). There was also an association between the presence of myofibroblasts and patient survival (Fig. 4). 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 few myofibroblasts; this difference was