Oral Oncology 47 (2011) 33–38

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Cancer-associated fibroblasts, a parameter of the tumor microenvironment, overcomes carcinoma-associated parameters in the prognosis of patients with mobile tongue cancer

Ibrahim O. Bello [a,1], Marilena Vered [b,c,1], Dan Dayan [b], Alex Dobriyan [c], Ran Yahalom [c], Kalle Alanen [d], Pentti Nieminen [e], Saara Kantola [a,f], Esa Läärä [g], Tuula Salo [a,f,*]

[a] Department of Diagnostics and Oral Medicine, Institute of Dentistry, University of Oulu, Oulu FI-90014, Finland
[b] The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel-Aviv University, Tel Aviv, Israel
[c] Department of Oral and Maxillofacial Surgery, The Chaim Sheba Medical Center, Tel Hashomer, Israel
[d] Department of Pathology, University of Turku, TYKS-SAPA-Liikelaitos, Hospital District of Southwest Finland, Finland
[e] Medical Informatics and Statistics Research Group, University of Oulu, Finland
[f] Department of Oral and Maxillofacial Surgery, Oulu University Hospital, Oulu, Finland
[g] Department of Mathematical Sciences, University of Oulu, Finland

ARTICLE INFO

Article history:
Received 12 October 2010
Received in revised form 26 October 2010
Accepted 26 October 2010
Available online 26 November 2010

Keywords:
Tongue cancer
Cancer-associated fibroblasts
Stromal myofibroblasts
Ki-67
DNA content
Ploidy
Maspin
Disease-specific mortality
Prognosis
Oral cancer

SUMMARY

Mobile tongue squamous cell carcinoma (MTSCC) is known for its strong propensity for regional metastasis and poor patient survival despite aggressive treatment, thus calling for new and reliable markers for predicting prognosis and guiding therapeutic management. Towards this end, three classes of markers were investigated: cancer-associated fibroblasts (CAFs; α-SMA positivity) as a representative of the tumor microenvironment, maspin (*ma*mmary *s*erine *p*rotease *in*hibitor) as a tumor marker likely to be modulated by factors within the tumor microenvironment, and DNA content and Ki-67 labeling index as inbuilt tumor markers in 128 cases of MTSCC using immunohistochemistry and image cytometry. Of these markers, only CAF density was independently and relatively strongly associated with elevated mortality from MTSCC. The hazard ratio in the CAF-rich type of tumor microenvironment was 4.85 (95% CI 1.41–16.6, versus the CAF-poor) when adjusted by proportional hazards modeling for the center where the patient was managed, gender, tumor stage, presence of neck metastasis and age at diagnosis. CAF density was unrelated to non-MTSSC mortality. Given the strong association between increased CAF density and higher mortality in MTSCC, routine assessment of CAF density for disease course prognosis and inclusion as an integral part of treatment protocols are recommended.

Introduction

The incidence and death rates of cancers of the oral cavity and pharynx have slightly decreased over the past few decades,[1,2] but the incidence of cancers of the tongue, oropharynx and tonsil are on the rise.[1] Furthermore, mobile tongue cancer is associated with poorer survival and a lower rate of local tumor control than other sub-sites with squamous cell carcinoma (SCC) of the head and neck region.[3] This raises the possibility that mobile tongue SCC (MTSCC) is biologically distinctive[4] and, as such, should be analyzed in separate prognostic studies, ideally, large-scale, multicenter and standardized ones.

Substantial evidence indicates that the development and progression of cancer not only depend on its genetic characteristics but also on interactions with its microenvironment, where tumor cells may alter the surrounding stroma and, in turn, stromal cells may promote cancer progression and acquisition of invasiveness.[5,6] Cancer-associated fibroblasts (CAFs) are an excellent example for the sequel of the former and the executive of the latter. CAFs were shown to have emerged concomitantly with SCC cells in both an animal model of tongue carcinogenesis[7] as well as in humans.[8] Their overall distribution within the tumor microenvironment (TME) was found to be related to disease recurrence.[9] A preliminary study on tongue SCC (without specifying whether it was mobile or base of tongue) reported that overall survival was related only to CAFs located at the invasive front but not to CAFs distributed throughout the entire tumor stroma.[10] The presence of CAFs in the TME of metastatic lymph nodes of MTSCC, in addition to their pair-matched primary tumors, further emphasizes the essential role that CAFs play in tumor growth, invasiveness and spread.[11]

* Corresponding author. Address: Department of Oral and Maxillofacial Surgery, Oulu University Hospital, Oulu, Finland. Tel.: +358 8 537 5592; fax: +358 8 537 5560.
E-mail address: tuula.salo@oulu.fi (T. Salo).
[1] These authors contributed equally to this work.

1368-8375/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.oraloncology.2010.10.013

Maspin (*ma*mmary *s*erine *p*rotease *in*hibitor) is considered as being a tumor suppressor factor that increases cell adhesion and apoptosis, and decreases motility, angiogenesis and pericellular proteolysis.[12] The clinical contribution of maspin in cancer progression and metastasis is complex and seems to be the product of a number of factors, such as the genetic background of the tumor, type of cancer, expression of maspin (or lack of it) in the normal counterpart tissue and subcellular distribution. Furthermore, it has been shown that changes in the expression of maspin are modulated by cancer-associated inflammation and its mediators.[13,14] Associations between the expression of maspin and clinical outcomes of patients with MTSCC are equivocal.[15,16]

In this study, a relatively large series of MTSCC patients was assembled from two centers. We investigated the density of CAFs (α-SMA positivity) in the TME, maspin (a tumor marker liable to be modulated by factors within the TME), and the DNA content and Ki-67 labeling index (each an inbuilt tumor marker), and analyzed the association of these factors with mortality from MTSCC and from other causes of death.

Materials and methods

Patients

Seventy-seven patients treated at the Department of Otorhinolaryngology, Oulu University Hospital between 1983 and 2005, and 51 patients treated at the Sheba Medical Center between 1981 and 2006[9] were eligible for the study. The inclusion criteria were sufficient clinical data and histological material for evaluation and comparison. All 128 patients had undergone primary resection and a concomitant neck dissection and had received no prior treatment for their MTSCC. Additionally, 49 also underwent radiotherapy, 10 radiotherapy and chemotherapy, and one chemotherapy.

Formalin-fixed, paraffin-embedded tissue blocks were obtained from the archives of Oulu University Hospital, Finland and the Institute of Pathology, Sheba Medical Center, Israel. The available 128 blocks were cut into 4-μm-thick sections and stained with α-SMA (clone 1A4, 1:100, Dako A/S, Denmark), maspin (clone EAW24, 1:75, Novocastra, Newcastle upon Tyne, UK), and Ki-67 (polyclonal, 1:50, Dako A/S, Denmark). Eight micrometer-thick sections were prepared for DNA ploidy analysis. All analyses were performed by at least two of the authors (IOB, MV, KA and TS) who were unaware of the clinical outcome at the time of analyses. The study was approved by the Ethical Committee of the Northern Osthrobothnia Hospital District, Finland and the Institutional Review Board, Sheba Medical Center, Israel.

Analyses of immunohistochemical staining

The α-SMA cases were graded according to the CAF density throughout the entire tumor stroma, similar to a previous description of this technique[9]: 4 = dense overlapping of CAFs distributed throughout the tumor predominantly of epithelioid morphology, with essentially no distinct border with the SCC, 3 = similar to grade 4, somewhat less dense, or CAFs not distributed throughout the entire tumor, 2 = predominantly spindle, less dense, usually a clear border between CAFs and the SCC, 1 = either spindle or epithelioid morphology of the CAFs in a focal pattern, and 0 = no detectable CAFs. Each tumor was scored according to the most severe grade. For statistical analysis, grades 0 and 1 were grouped as CAF-poor, grades 2 and 3 as CAF-medium, and grade 4 as CAF-rich.

The maspin cases were analyzed by scoring the intensity as 0 = none, 1 = weak, and 2 = strong. The quantity of maspin was scored from 0 to 1. It was possible for a single tumor to have all three intensity patterns, and each was multiplied by its corresponding quantity, after which all of the scores added together for a final score. The maximum value was therefore 2 × 1.0 for a tumor of maximum intensity and with all of its cells having been stained. Maspin categorization was 0.4–1.2 (low), 1.3–1.6 (medium) and 1.7–2.0 (high).

The Ki-67 cases (only patients from Oulu) were analyzed, and labeling indices (LIs) were calculated as previously described.[16] Categorization of the Ki-67 LI values (based on approximate tertiles was low (13.4% to 31.4%), medium (31.5% to 40.4%) and high (>40.4%).

Static cytometry

Eight-micrometer-thick sections (77 cases from Oulu and 10 from Tel Aviv) were cut on charged glass slides and deparaffinized. In all, 67 cases had interpretable histograms (Table 1). No enzymatic disintegration was done. Modified Feulgen staining, static (image) cytometric measurements and histogram classification were carried out as previously described.[17] At least 30 muscle cells or fibroblasts were measured within each section to serve as internal controls.

Statistical analysis

The prognosis of the patients in relation to the three selected immunohistological markers and to DNA ploidy was analyzed in

Table 1
Distribution of patients (n = 128) by demographic and clinicopathologic factors. The figures refer to the n (%) of patients, unless otherwise stated.

	All patients (n = 128)	Oulu (n = 77)	Tel Aviv (n = 51)
Sex			
Male	60 (47)	34 (44)	26 (51)
Female	68 (53)	43 (56)	25 (49)
Age (years)			
20-49	27 (21)	13 (17)	14 (28)
50-64	36 (28)	21 (27)	15 (29)
65-79	49 (38)	30 (39)	19 (37)
80+	16 (13)	13 (17)	3 (6)
Range	20–99	26-99	20-80
Median	65.0	68.0	62.0
Stage			
I/II	70 (55)	36 (47)	34 (67)
III/IV	55 (43)	38 (49)	17 (33)
Unknown	3 (2)	3 (4)	
Neck metastasis			
Present	38 (30)	23 (30)	15 (29)
Absent	84 (65)	48 (62)	36 (71)
Missing	6 (5)	6 (8)	0 (0)
CAF (stromal MF) score			
Poor (0–1)	35 (27)	17 (22)	18 (35)
Medium (2–3)	62 (49)	44 (57)	18 (35)
Rich (4)	31 (24)	16 (21)	15 (30)
Maspin score			
Low (0.4–1.2)	37 (29)	18 (23)	19 (37)
Medium (1.3–1.6)	38 (30)	28 (37)	10 (20)
High (1.7–2.0)	42 (33)	23 (30)	19 (37)
Missing	11 (8)	8 (10)	3 (6)
DNA content			
Diploid	13 (10)	12 (16)	1 (2)
Aneuploid	44 (34)	40 (52)	4 (8)
Tetraploid	10 (8)	10 (13)	0 (0)
Missing	61 (48)	15 (19)	46 (90)
Ki-67 (%)			
Low (13.4–31.4)	19 (15)	19 (25)	–
Medium (31.5–40.4)	19 (15)	19 (25)	–
High (40.5–62.7)	18 (14)	18 (23)	–
Missing	72 (56)	21 (27)	51 (100)