ELSEVIER

Auris Nasus Larynx 37 (2010) 77–83

AURIS NASUS LARYNX
INTERNATIONAL JOURNAL OF ORL & HNS

www.elsevier.com/locate/anl

Clinicopathologic significance of CD105-assessed microvessel density in glottic laryngeal squamous cell carcinoma

Elvir Zvrko [a,*], Anton Mikic [b], Ljiljana Vuckovic [c]

[a] *Clinic for Otorhinolaryngology and Maxillofacial Surgery, Clinical Center of Montenegro, Podgorica, Montenegro*
[b] *Institute for Otorhinolaryngology and Maxillofacial Surgery, School of Medicine, University of Belgrade, Belgrade, Serbia*
[c] *Center for Pathology and Forensic Medicine, Clinical Center of Montenegro, Podgorica, Montenegro*

Received 28 December 2008; accepted 20 May 2009
Available online 23 June 2009

Abstract

Objective: Intratumoral microvessel density (MVD) determined with the use of antibodies to endoglin (CD105) is considered to be an important prognostic marker in a variety of malignancies. The purpose of this study has been to analyze the clinicopathologic significance of CD105-assessed MVD in SCCs primary localized in glottic region of larynx.
Methods: Surgical specimens from 40 patients with resected glottic squamous cell carcinomas were immunostained for CD105. CD105-assessed MVD was calculated at 400× magnification. Using the mean MVD as a cut-off, tumors were classified in the ''high MVD'' group and in the ''low MVD'' group. Clinicopathologic data were collected retrospectively.
Results: The mean MVD assessed by CD105 in considered glottic SCCs was 12.3 (standard deviation [SD] = 3.65). MVD varied among tissue samples from 5 to 21 (median 12.5). High MVD was significantly correlated with a more aggressive tumor phenotype, including T3–T4 tumor (Fisher exact test, $P = 0.004$) and advanced clinical stage (Fisher exact test $P = 0.026$). Kruskal–Wallis test identified significant relation between pT stages and CD105-assessed MVD ($P = 0.011$). CD105-assessed MVD was significantly related to malignancy recurrence presence/absence (Mann–Whitney *U*-test $P = 0.023$). Logistic regression in multivariate modality showed that MVD (odds ratio [OR] 2.29, $P = 0.033$, 95% confidence interval [CI] 1.06–7.53) and advanced T category (T3–T4) (OR 4.11, $P = 0.026$, 95% CI 2.38–9.46) were significantly related to malignancy recurrence presence/absence. Cox regression analysis revealed that expression of CD105 ($P = 0.031$) and N status ($P = 0.014$) were the independent factors for disease-free survival.
Conclusion: High expression of CD105 correlated significantly with advanced T status and locoregional recurrence. The present preliminary results suggest that CD105-assessed MVD in primary glottic squamous cell carcinomas may identify patients at risk of disease recurrence.

Keywords: Angiogenesis; CD105; Mean vascular density; Laryngeal carcinoma; Prognosis

1. Introduction

Angiogenesis, or neo-vascularization, is the term used for the growth and development of new capillary vessels. Tumor angiogenesis, the formation of peritumor and intratumor new blood vessels, is necessary to the growth and metastasis of solid tumors by supplying nutrient and oxygen, disposing metabolites and releasing growth factors that promote tumor cell proliferation, tumor progression in the host stroma and metastasis development [1]. Solid tumors cannot grow beyond 1–2 mm in diameter without angiogenesis [2]. Angiogenesis is a complex multistep process involving extracellular matrix remodeling, endothelial cell migration and proliferation, microvessel differentiation and anastomosis. These processes are tightly controlled by positive and negative angiogenic factors and their receptors that regulate one or more of these key events [3,4]. However, the regulatory mechanisms in angiogenesis are not yet fully understood.

Intratumoral microvessel density (MVD) is considered a promising prognostic marker in a variety of malignancies and increased MVD correlated with malignancy progression

* Corresponding author at: Ljubljanska bb, Clinical Center of Montenegro, 81000 Podgorica, Montenegro. Tel.: +382 69 401 431.
E-mail address: elvirz@t-com.me (E. Zvrko).

0385-8146/$ – see front matter
doi:10.1016/j.anl.2009.05.005

and shorter overall and relapse-free survival rates. Intratumoral MVD has been previously studied using panendothelial markers such as CD34, CD31 and Factor VIII, which cannot distinguish proliferating endothelium in tissue undergoing angiogenesis from normal pre-existing blood vessels. In contrast to panendothelial antibodies, endoglin (CD105) antibodies have shown a greater specificity for tumor vasculature. Endoglin is a 180-kDa homodimeric type-I transmembrane glycoprotein that modulates transforming growth factor-β (TGF-β) signaling by interacting with TGF-β receptors I and III [5]. The gene encoding for CD105 is approximately 40 kb long and it has been mapped to human chromosome 9q34 [6]. Li et al. [7] demonstrated that hypoxia activates the CD105 gene promoter, augmenting its mRNA transcription and protein translation. Several studies have defined the role of CD105 as a powerful marker to quantify MVD in solid and hematopoietic tumors including breast cancer [8,9], cutaneous melanoma [10], colon cancer [11–13], esophageal cancer [14], lung cancer [15,16], gynecological malignancies [17,18], renal cancer [19], head and neck cancer [20–24] and in multiple myeloma [25] and hairy cell leukemia [26].

To the best of our knowledge, only a few studies investigated endoglin expression in laryngeal squamous cell carcinomas (SCCs). The purpose of the present study has been to analyze for the first time the clinicopathologic significance of MVD in SCCs primary localized only in glottic region of larynx using anti-CD105 monoclonal antibody.

2. Materials and methods

2.1. Patients

Medical charts of patients who had been treated for primary laryngeal SCCs at the Clinic for Otorhinolaryngology and Maxillofacial Surgery of the Clinical center of Montenegro in Podgorica from 2003 to 2008 were reviewed. The clinical information, including sex, age, histologic and nuclear grade, primary tumor (T) classification, nodal (N) status, TNM stage, and oncological outcome, were obtained retrospectively from clinical records. In the analysis of clinical data we have defined poor oncological outcome as recurrence of disease or occurrence of metastasis after treatment.

Forty cases of laryngeal invasive SCCs localized in glottic region with available follow-up data were evaluated, 20 of them had early cancer (stage I or II) and 20 had advanced cancer (stage III or IV). All selected patients underwent complete resection as primary treatment. Patients with second primaries or who had received primary radiotherapy and/or chemotherapy were not considered. Treatment decision-making was based on clinical stage and on the presence or not of lymph node metastasis at the time of diagnosis. Partial laryngectomy was performed in 22 and total laryngectomy in 18 patients. Nine patients received neck dissection operation simultaneously to the primary tumor removal and lymph node metastasis was presented in four cases. Eight patients underwent postoperative radiotherapy. Mean follow-up time (calculated in months from treatment completion to the last otolaryngological control) was 20.5 months (range 6–60 months). Pathological staging was determined according to the 6th TNM Classification of Malignant Tumors of the International Union Against Cancer. Clinicopathologic characteristics of the selected patients are shown in Table 1.

Table 1
Clinicopathologic characteristics of 40 patients with glottic squamous cell carcinoma.

	No. of patients	(%)
Sex		
Male	29	(72.5)
Female	11	(27.5)
T stage		
T1	12	(30)
T2	10	(25)
T3	16	(40)
T4	2	(5)
N stage		
N0	36	(90)
N1	3	(7.5)
N2	1	(2.5)
TNM stage		
I	12	(30)
II	8	(20)
III	18	(45)
IV	2	(5)
Histological grading		
G1	23	(57.5)
G2	17	(42.5)
Nuclear grading		
G1	14	(35)
G2	24	(60)
G3	2	(5)

2.2. Immunohistochemistry

Forty tissue blocks of glottic SCCs were fixed in 10% formalin and embedded in paraffin wax. All included samples originated from complete resection material. We selected the best section from each block showing central and peripheral areas of the tumor, avoiding areas with necrosis. One 4-mm-thick section from each block was cut and deparaffinized. The sections were pretreated with Proteinase K for 5 min. The endogenous peroxides' activity was blocked with H_2O_2 solution in methanol for 10 min. The slides were incubated with mouse monoclonal antibody CD105 (clone SN6h diluted 1:20, DAKO, Denmark) and washed with TRIS-buffered saline. Diaminobezidine (DAB) was used as a chromogen. The slides were then counterstained with hematoxylin. Laryngeal tissues free of tumor