

Investigation of Biomarker in Laryngeal Carcinomas

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Background: The aim of the study is to determine whether there is a role of *podoplanin* and glutathione S-transferases T1 (GST-T1) expression in laryngeal squamous cell carcinoma. **Methods:** In this study, 33 patients were enrolled and gene expression analysis was performed by qRT-PCR. The *podoplanin* and GST-T1 expression patterns were analyzed to determine their correlation with clinicopathologic parameters of laryngeal cancer. **Results:** Of all included patients, 20 had supraglottic, and 13 had glottic laryngeal cancer. Increased expression of *podoplanin* was found in seven

(35%) supraglottic tumor tissues and seven (53.8%) glottic tumor tissues, but GST-T1 expression was not detected. **Conclusion:** *Podoplanin* expression did not show any prediction for tumor differentiation, regional metastasis, thyroid cartilage invasion, lymphatic vessel invasion, or tumor differentiation for laryngeal cancer, and also there were no significant differences in *podoplanin* expression between glottic and supraglottic regions, but extracapsular extension is almost statistically significance ($P = 0.05$). J. Clin. Lab. Anal. 28:186–190, 2014. © 2014 Wiley Periodicals, Inc.

Key words: *Podoplanin*; GST-T1; laryngeal carcinoma; biomarker; squamous cell carcinoma

INTRODUCTION

Laryngeal cancer is the only cancer type among all malignancies for which the survival rate decreased in the last decade. Most of the laryngeal tumors are malignant and 95–98% are squamous cell carcinomas (SCC) (1). Human *podoplanin* gene, which consists of 162 amino acids, is a 38 kDA mucin-type transmembrane glycoprotein and localized in 1p36.21. As *podoplanin* is expressed especially in lymphatic endothelial cells, it is not expressed in endothelium of blood vessels (2). *Podoplanin* is an intracellular protein that is reported to be expressed in lymphatic endothelium, alveolar type-I cells, osteoblasts, and

peritoneal mesothelial cells. It is not expressed in normal vascular endothelial cells (3, 4). *Podoplanin* also plays an important role in peripheral lung cell proliferation regulation and lymphatic vascular development. The *podoplanin* expression is upregulated in many different human cancers including squamous cell carcinomas of the oral cavity, lung, cervix, esophagus, and skin and also in dysgerminomas of the ovary and granulosa cell tumors, breast tumors, colorectal tumors, melanomas, mesotheliomas, and some tumors of the central nervous system (CNS) (5). Increased expression of *podoplanin* may cause a higher rate of lymph node metastasis. In addition, patients with lymph node metastasis and upregulated *podoplanin* expression had shorter disease-specific survival rate than other patients. According to epidemiologic data, 25% of cases have regional and 8–10% have distant metastasis (6). *Podoplanin* is frequently expressed in cutaneous head and neck squamous cell carcinoma (HNSCC) and may serve as a predictor for regional lymph node metastasis, locoregional recurrence, and clinical outcome (7). Neck metastasis is one of the most valuable prognostic factor

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Received 4 January 2013; Accepted 24 June 2013

DOI 10.1002/jcla.21664

Published online in Wiley Online Library (wileyonlinelibrary.com).

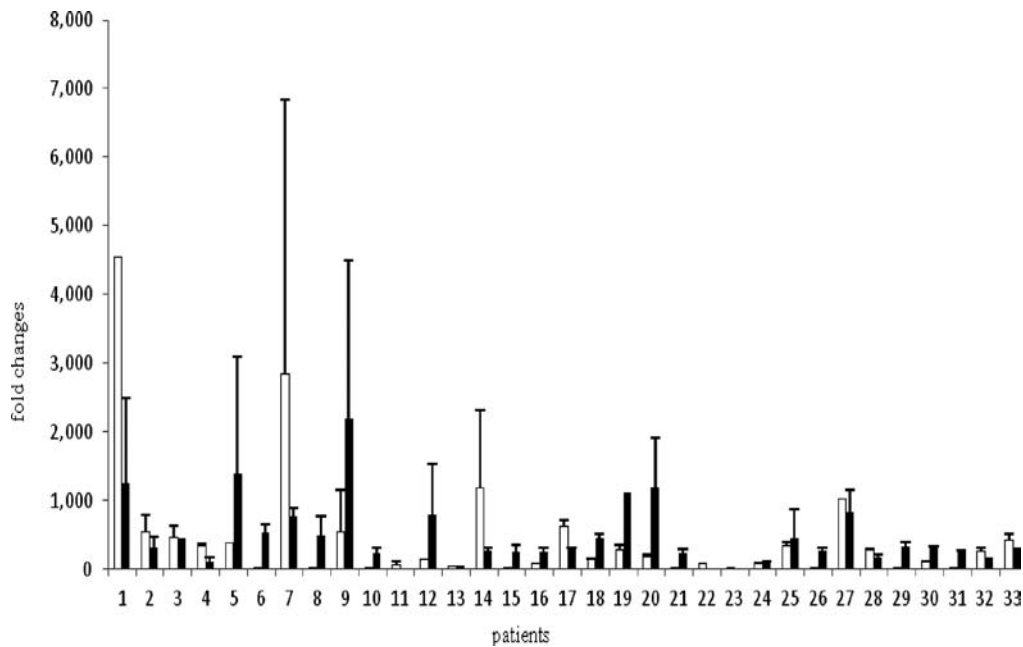


Fig. 1. *Podoplanin* expression in laryngeal cancer patients.

of survival rate. Laryngeal SCC tumor stages and localizations may have different neck metastasis patterns. This may be due to the molecular structure and the biological behavior of the tumor. Regional metastasis may be related with lymphangiogenesis. Treatment varies according to the tumor stage and localization. Glottic cancers have better survival rates than supraglottic and subglottic cancers. Five-year survival rates change between 65.7 and 88.6% (8, 9). The most common reason of mortality due to laryngeal SCC is the locoregional recurrence.

The glutathione S-transferases (GSTs) is an important family of enzymes involved in phase-II xenobiotic metabolism that catalyze biosynthesis and metabolism of many substances including detoxification of exogenous chemical carcinogens, such as aromatic polycyclic hydrocarbons present in tobacco (10). GST-T1 enzyme, in GST-T class with its gene is, located on chromosome 22q11.2 (11). It has been shown that individuals carrying the null genotype of GST have significantly reduced activity of this antioxidant enzyme (12, 13) and so have higher levels of intermediates of oxidative metabolism. This genotype is related with many diseases (14). The revealed alterations in expression of *GST-T1* enzyme can cause activation of carcinogenic particles or extinction of toxic effects. Therefore, GST-T1 enzyme can be used as an important biomarker for diagnosis of laryngeal cancer.

Thirty-three patients (all males) with mean age \pm SD of 58.03 ± 11.10 years, underwent histopathological examinations and total or partial laryngectomy operation with or without neck dissection in Istanbul University Faculty of Medicine Department of Otorhinolaryngology—Head

and Neck Surgery, were included to the study (between November 2010 and November 2011). The patients who received primary other therapies, such as radiotherapy or chemotherapy, for laryngeal cancer were excluded. Tissue samples were obtained from both healthy adjacent mucosa and the tumor tissue itself during the surgery.

Total RNA was extracted from the tissue samples using Roche, High Pure RNA Tissue Kit (Cat. no. 12033674001 Roche, GmbH) according to the manufacturer's instructions. RNA samples were quantified using a NanoDrop[®] ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). First strands of the cDNA samples were synthesized using RT PCR (Cat. no. 11483188001 Roche). The β -*Actin* (*ACT* β) gene was used as reference for normalization of the gene expression levels.

Podoplanin overexpression was found in 14 patients on the other hand, decreased *podoplanin* expression was found in 19 patients (Figure 1). *Podoplanin* expression did not show significant difference for tumor differentiation, regional metastasis, thyroid cartilage invasion, lymphatic vessel invasion, tumor stage tumor localization, N stage for laryngeal cancer, but extracapsular extension was almost statistically significant ($P = 0.05$). The association between the patient characteristics and their *podoplanin* expressions is shown in Table 1.

These findings suggest that *podoplanin* plays a role in the progression of epithelial cancers. The physiological function of *podoplanin* is still uncertain. This situation inspires the investigators to find biological markers to predict the tumoral behavior. *Podoplanin* expression was investigated in intratumoral and peritumoral tissues of patients with

TABLE 1. Association Between *Podoplanin* Expression and Clinicopathological Data of Patients

Characteristic	Podoplanin expression				P value
	Downregule (n = 19)		Upregule (n = 14)		
	N	%	N	%	
Age					0.622
Mean ± SD	54.89 ± 11.18		62.28 ± 9.817		
Median	55		61		
Smoking					1.00 ^a
Yes	18	58.1	13	41.9	
No	1	50	1	50	
Alcohol					0.561 ^a
Yes	1	33.3	2	66.7	
No	18	60	12	40	
Tumor differentiation					0.948
Well	1	50	1	50	
Moderately	17	58.6	12	41.1	
Poorly	1	50	1	50	
Tumor localization					0.284
Supraglottic tumor	13	65	7	35	
Glottic tumor	6	46.2	7	53.8	
Regional lymph node metastasis					0.241 ^a
Yes	7	77.8	2	22.2	
No	12	50	12	50	
Tumor stage					0.341
T1	0	0	2	100	
T2	8	61.5	5	38.5	
T3	8	66.7	4	33.3	
T4	3	50	3	50	
N stage					0.416 ^a
N0	13	52	12	48	
N1–3	6	75	2	25	
Extra-capsular spread of the lymph nodes					0.05 ^a
Yes	5	100	0	0	
No	14	50	14	50	
Thyroid cartilage invasion					0.212
Yes	4	40	6	60	
No	14	63.6	8	36.4	
Lymphatic vessel invasion					0.486
Yes	8	66.7	4	33.3	
No	11	52.4	10	47.6	

^aFisher's exact test.

tongue cancer. Rodrigo et al. found that *podoplanin* expression was related with regional metastasis, which is also supported by our study (15). However, any statistically significant difference about the tumor site was not found. Regional lymphatic metastasis was observed to be twofold higher in patients with low *podoplanin* expression level than in patients with high *podoplanin* expression level; but any statistically significant difference was not determined. *Podoplanin* expression levels vary considerably in dysplastic laryngeal epithelium tissue. Therefore, tissue culture should be observed in multiple regions instead of one region in some cases. Yuan et al. showed that patients, whose tumors expressed high lev-

els of *podoplanin*, had a statistically significant higher rate of lymph node metastasis (6). In addition, patients with lymph node metastasis and increased *podoplanin* expressions had shorter disease-specific survival rate than other patients. Kawaguchi et al. concluded that *podoplanin* was involved in oral tumorigenesis and may serve as a predictor for lymph node metastasis and poor clinical outcome (16). As known, the most prognostic factor of laryngeal cancers is regional lymphatic metastasis. Regional metastasis may be related with lymphangiogenesis. For this reason, we checked if *podoplanin*, expressed on lymphatic vessels but not on the capillary vessels, can be used for the prediction of regional metastasis. *Podoplanin*

expression levels revealed that patients with a significantly poor prognosis in SCC of hypopharynx did not show a significant shorter survival in SCC of laryngeal. Rodrigo et al. showed that the expression of *podoplanin* in the dysplastic lesions was correlated with the risk of progression to laryngeal cancer (15). The exact molecular function of cancer cell expressing *podoplanin* is currently studied (17). Recent data from studies of various human cancer types suggest a possible association of *podoplanin* expression with invasion and metastasis of tumors (18). *Podoplanin* expressions significantly decreased as the tumor classification levels increased. Therefore, it was proposed that the *podoplanin* expression may play a role at the initiation, but not in the progression, of laryngeal cancers. Moreover, no relationship was found between the *podoplanin* expression, the regional nodal metastasis, and tumor stage. In this study, extracapsular extension is almost statistically significant ($P = 0.05$). It is well known that supraglottic and glottic compartments of the laryngeal cancer were developed from different embryologic origin. Glottic region carcinomas are generally well-differentiated, supraglottic region carcinomas are moderate, and epidermoid carcinomas are poorly differentiated. Glottic region carcinoma spreads to anterior commissure with anterior extension, it also spreads to ventricular wall of supraglottic region with superior extension. Thus, extracapsular extension is an important marker for prognosis. Therefore, supraglottic area is rich in lymphatic vessels but glottic area has less lymphatic vessels. Rodrigo et al. showed higher levels of *podoplanin* expression in glottic carcinomas ($P = 0.01$) (15). On the other hand, in our study the increase of *podoplanin* expression was found to be higher in supraglottic carcinomas than in glottic carcinomas. The increase of *podoplanin* expression was obtained in early stages in patients with supraglottic carcinomas (35%) than in patients with glottic carcinomas (53.8%). The reason of the difference may be caused by the high levels of lymphatic duct's plexus localization in supraglottic carcinoma versus glottic carcinoma. Recent experimental results have demonstrated that *podoplanin* mediates a pathway leading to collective cell migration and invasion in vitro (19, 20). However, thyroid cartilage invasion depends directly on the primary tumor stage. In addition, extra capsular spreading of the nodal metastasis is related with tumor stage and survival rate.

Many biomarkers were found to determine the prognosis or metastatic disease of several malignancies, but no biological marker has been found for determination of the survival rate or metastatic disease for laryngeal cancer. GST-T1 expression was not detected, increased expression of *podoplanin* was found in 14 tumor tissues; but there was no significant difference in *podoplanin* expression between tumor tissue and normal tissue.

ACKNOWLEDGMENTS

We acknowledge the help of Ms. Allison Eronat and Ms. Kadriye Kahraman in executing this manuscript. We thank the Research Council of Istanbul University for supporting this study.

CONFLICT OF INTEREST

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

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