Original paper

Predictive value of ALDH1 and CD44 positivity for radiotherapy response and prognosis in early-stage glottic laryngeal squamous cell carcinoma

Ozgur Surmelioglu[1], Kivilcim Eren Erdogan[2], Muhammed Dagkiran[1], Suleyman Ozdemir[1], Ozgur Tarkan[1], Aysun Uguz[2]

[1]Department of Otorhinolaryngology, Faculty of Medicine, Cukurova University, Adana, Turkey
[2]Department of Pathology, Faculty of Medicine, Cukurova University, Adana, Turkey

To evaluate the predictive value of CD44 and aldehyde dehydrogenase 1 (ALDH1) expression for prognosis and radiotherapy (RT) response in patients with early-stage laryngeal cancer receiving RT.

Forty-four patients with early-stage laryngeal cancer diagnosed between 2002 and 2016 were included in the study. The correlation between RT response and pre-treatment immunohistochemical ALDH1 and CD44 staining was evaluated. In addition, survival times were compared between groups.

The mean age of the 44 patients was 59.8 ±9.0 (43-81) years and 41 were male. There were 20 patients in the non-recurrent group (all men) and 24 patients in the recurrent group (21 men). Immunohistochemical positivity for ALDH1 was found to be a significant risk factor for RT failure ($p = 0.0001$), whereas CD44 positivity ($p = 0.114$) and age group ($p = 0.287$) were not significant.

ALDH1 positivity was identified as a significant predictor of DFS and RT sensitivity, while CD44 positivity did not differ according to RT response.

Key words: laryngeal squamous cell carcinoma, ALDH1, CD44, radiotherapy.

Introduction

Although new treatment protocols for head and neck cancer have been developed in recent years, head and neck cancer is still the sixth most common cancer worldwide, mainly due to its association with human papillomavirus (HPV) and tobacco and alcohol usage [1]. The most common histopathological type is squamous cell carcinoma (SCC), and the oral cavity, larynx, and pharynx are most frequently involved [2].

Laryngeal SCC is the most common head and neck cancer, affecting an estimated 100,000 people per year [3]. For patients who present with advanced disease, the mainstay of treatment is total laryngectomy with or without adjuvant therapy [4]. However, the survival rate is low. Among laryngeal cancers, glottic carcinomas are the most common subgroup, with the glottis being involved approximately 3 times more often than the supraglottic larynx. Glottic cancers are usually diagnosed in the early stage due to symptoms of hoarseness.

Early-stage laryngeal cancers, inclusive of T1-2/N0 disease, are treated successfully with a single, locally directed treatment modality. If the tumor has grown deeper, but it is only in the vocal cords, and they move normally it is called T1, and if the tumor has grown into the supraglottis or subglottis, and/or the vocal cords move normally it is called T2 tumor. If there is no lymph node involvement in both cases, these tumors are called early stage glottic carcinoma. In early glottic cancer, lymph node metastasis

is rarely seen, with an incidence of clinically positive lymph nodes of nearly zero for stage T1 and < 2% for stage T2 disease, and a complete cure can often be achieved by radiotherapy (RT) or surgery [5-7]. Therefore, the goal is to achieve the best local control leading to a complete cure and optimal functional results. At present, there are various treatment modalities for treating early glottic cancer; namely, RT or partial laryngectomy techniques. Although surgery has been used for decades, its use has greatly decreased in recent years because of declining functional results and advances in RT [8]. The optimal treatment for early glottic cancer has remained an issue of debate, primarily due to a lack of evidence from large prospective randomized trials [9]. Recent studies have shown similar local control between RT and surgery. Mendenhall *et al.* reported local control rates ranging from approximately 80% to 94% for T1 tumors and 70% to 85% for T2 tumors with both modalities [10, 11].

Several prognostic factors can be used for the evaluation of laryngeal cancer. Microscopic grade is an independent prognostic factor and correlates with clinical stage [12]. Recurrence is related to aneuploidy [13]. The presence of S100-positive Langerhans cells around the tumor is called the host reaction and has been associated with a favorable prognosis [14].

The most accepted prognostic factors are the TNM classification. However, the TNM system cannot distinguish aggressive tumors from nonaggressive tumors of the same size. Identifying one or more biomarkers to predict the biological behavior of head and neck squamous cell carcinomas (HNSCCs) would be beneficial. Recently, a small population of cancer cells referred to as cancer stem cells (CSCs) was found to be responsible for tumor initiation, relapse, and resistance to chemotherapy or RT; therefore, eradicating CSCs is considered critical in cancer therapy [15, 16]. The CSC hypothesis has also been proposed for HNSCCs; some cell surface markers have been reported as CSC markers in HNSCC, such as CD44, CD133, ALDH1, and ABCG2 [17-19], and high expression of these markers is usually regarded as an indicator of poor prognosis. Among them, CD44 is the most reported CSC marker in HNSCC [20-22].

In this study, we aimed to evaluate the predictive value of CD44 and ALDH1 expression for prognosis and treatment response in patients with early-stage laryngeal cancer receiving RT.

Materials and methods

Forty-four patients diagnosed as having early-stage laryngeal cancer and treated with RT in the Department of Otorhinolaryngology, Faculty of Medicine of the Cukurova University between 2002 and 2016 were included in the study. Ethics committee approval of the study was obtained from the ethics committee of the Cukurova University (approval number-March 3,2017;62/27). Patients were treated with curative radiotherapy with a linear accelerator with a peak energy of 6 megavoltage (MV). All patients were treated with 225 cGy/28 days in the same radiotherapy center. The demographic and clinical data, pathology reports, prognostic parameters, and survival rates of the patients were retrieved retrospectively from their medical records. Among patients with early-stage disease, patients with recurrent disease and cured were studied with the same sampling method. Patients with anterior commissure involvement were excluded from the study. The patients were divided into two groups, those who showed complete response with RT (non-recurrent group) and those who developed local recurrence despite RT (recurrent group). The correlation between treatment response and immunohistochemical staining for ALDH1 and CD44 at the time of diagnosis was evaluated. Survival rates were also compared between the groups.

Histopathological analysis

For each patient, slides stained with hematoxylin-eosin were reviewed by the same pathologist, tumor tissues were selected, and ALDH1 and CD44 immunohistochemistry was performed on these tissues. For immunohistochemical staining, 4-micron thick sections were obtained from paraffin-embedded blocks in positively charged glasses and then deparaffinized. Rehydration, blockade with hydrogen peroxide, and 20 minutes of antigen retrieval were performed with sodium citrate buffer in a microwave. Samples were incubated overnight at 2-8°C with anti-ALDH1 rabbit monoclonal antibody (ab9883, Abcam) (1:500) (5 μg/ml) and CD 44 antibody (1:50) (Santa Cruz). After incubation with antibody, samples were stained with DAB chromogen. Finally, all samples were stained with Mayer hematoxylin and washed with distilled water and PBS.

For immunohistochemical analysis, a semiquantitative evaluation was performed. The intensity and extent of staining were scored for ALDH1. Density was scored as 0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining. Extent of staining was evaluated based on the percentage of positive cells. A total score was obtained by adding the density and extent scores. The evaluation of CD44 staining was based on membranous staining and was performed using the same scoring method.

Statistical analyses

The χ^2 test or Fisher's exact test was used to compare the groups. The Kaplan-Meier method and Cox proportional regression model were used to estimate the mean/median survival rates, failure rates, and

hazard ratios (HRs). The log-rank test was used to compare the survival distributions between groups. The prognostic ability of ALDH1 and CD44 were evaluated for DFS in both univariate and multivariable analyses. DFS was defined as the time from diagnosis to any documented clinical progression, relapse, or death from any cause. The results were reported as mean ±SD, median, number (n) and percent (%). A p value < 0.05 was considered significant. The analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY).

Results

Forty-four patients with early-stage laryngeal cancer and treated with RT were retrospectively evaluated. Their mean age was 59.8 ±9.0 (43-81) and 41 were men. Before treatment, all patients were confirmed as having early-stage disease according to the findings of indirect laryngoscopic and radiologic evaluation and were reported as SCC according to pre-treatment biopsy. Tumor grade was I in 19 patients (9 non-recurrent group/10 recurrent group), II in 12 patients (5 non-recurrent group/7 recurrent group) and III in 13 patients (6 non-recurrent group/7 recurrent group). Thirty-four patients had T1 glottic tumor and 11 had T2 glottic tumors. There was no anterior commissure involvement seen in any patients. All of the patients were diagnosed with early-stage glottic carcinoma according to the classification system of AJCC 2017 [23].

There were 20 patients (all men) in the non-recurrent group (15 patients were diagnosed as T1 and 5 T2 according to the classification system of AJCC 2017) and 24 patients (22 men and 2 women) in the recurrent group (18 patients were diagnosed as T1 and 6 T2 according to the classification system of AJCC 2017). There was no statistically significant difference between tumor grade and recurrence of disease. Median follow-up time was 48 months (22-88 months). Treatment failure was observed in 24 cases. Table I shows the demographic and clinical features of the patients.

Table II shows demographic and clinical features of the patients according to treatment response. While positive ALDH1 staining ($p = 0.0001$) was found to be significant risk factor for treatment failure, positive CD44 staining ($p = 0.114$) and age group ($p = 0.287$) were not significant factors.

Means and medians for disease-free survival (DFS) time are shown in Table III. The mean OS was 60.7 months (median: 62.0 months). The cumulative proportion of surviving patients was 81% at 12 months (1 year), 56% at 24 months (2 years), and 42% at 36 months (3 years). DFS was shorter in cases with positive ALDH1 staining and CD44 staining ($p = 0.0001$); Fig. 1A shows DFS times according

Table I. Demographic and clinical features of patients

Parameter	n	%
Age groups (years)		
0-55	14	31.8
56+	30	68.2
Gender		
Male	41	93.2
Female	3	6.8
Response to RT		
CR	20	45.5
TF	24	54.5
Salvage surgery		
TL	19	43.2
PL	5	11.4
RT	20	45.5
ALDH1 staining		
Negative	21	47.7
Positive	23	52.3
CD44 staining		
Negative	41	93.2
Positive	3	6.8
	Mean ±SD	Median (min-max)
Age (years)	59.8 ±9.0	59 (43-81)
Follow-up time (months)	52.1 ±20.5	48 (22-88)
ALDH1 total score	1.25 ±2.11	0 (0-7)
CD44 total score	0.25 ±0.83	0 (0-4)

RT – radiotherapy; CR – complete response; TF – treatment failure; TL – total laryngectomy; PL – partial laryngectomy; SD – standard deviation

Table II. Demographic and clinical features of patients according to prognosis status

	Response to Radiotherapy				
	Complete response		Treatment failure		
	n	%	n	%	p value
Age group					
0-55	8	57.1	6	42.9	0.287
56+	12	40.0	18	60.0	
ALDH1 staining					
Negative	19	90.5	2	9.5	0.0001
Positive	1	4.3	22	95.7	
CD44 staining					
Negative	20	50.0	20	50.0	0.114
Positive	0	0.0	4	100.0	

Table III. Means and medians for disease-free survival (DFS) time

	Mean DFS (months)	Median DFS (months)	Number of events/ censored	Percent censored	p
Age groups					
0-55	68.6	80.0	8/6	57.1	
56+	57.2	48.0	18/12	40.0	0.187
Gender					
Male	61.4	69.0	21/20	48.8	
Female	58.3	62.0	0/3	0.0	0.437
ALDH1 staining					
Negative	82.8	-	2/19	90.5	
Positive	45.8	39.0	22/1	4.3	0.0001
CD44 staining					
Negative	63.4	69.0	20/20	50.0	
Positive	35.2	24.0	4/0	0.0	0.001
Overall	60.7	62.0	44/24	45.5	

Table IV. Results of Cox regression model

	B	HR	95% CI	p
Age groups (>55 years)	0.71	2.0	0.7-5.5	0.167
ALDH1 positivity	2.80	16.4	3.6-73.5	0.0001
CD44 positivity	0.62	1.9	0.5-5.8	0.284

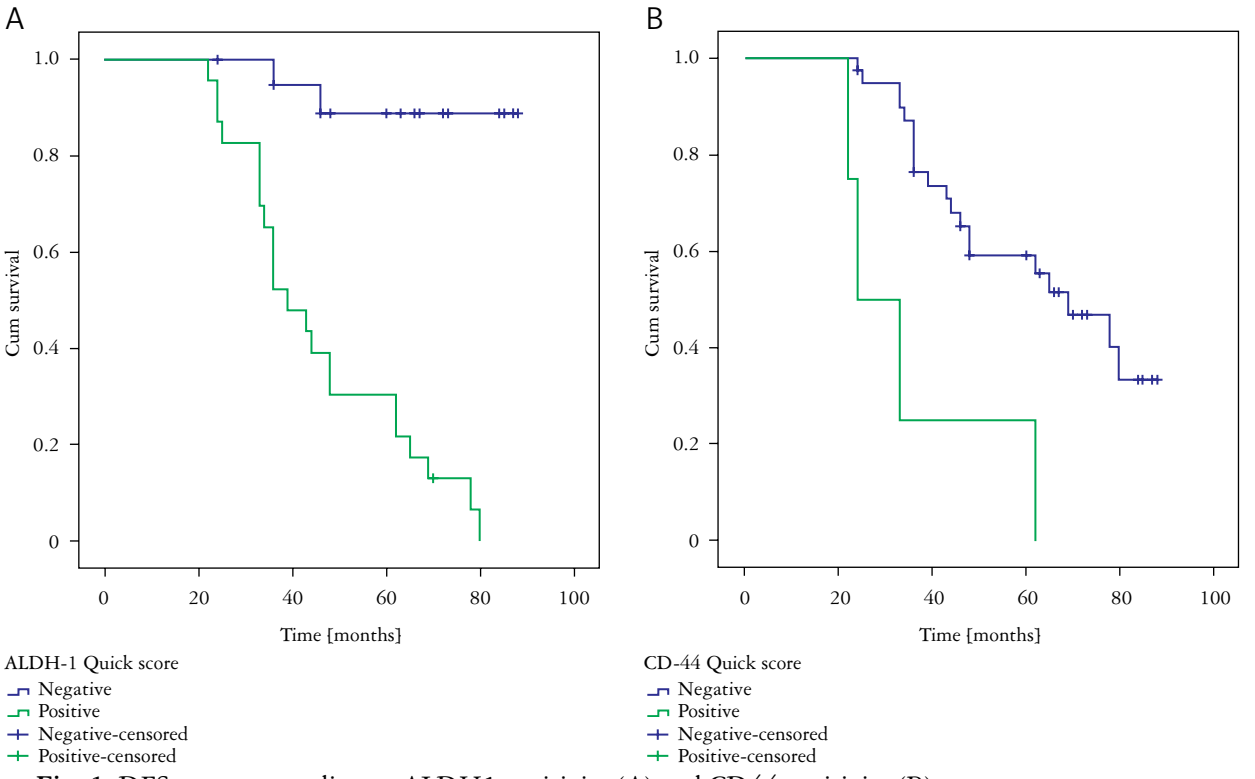

Fig. 1. DFS curves according to ALDH1 positivity (A) and CD44 positivity (B)

to ALDH1 positivity and Fig. 1B shows DFS times according to CD44 positivity.

Table IV shows the results of the Cox regression analyses. Two Cox regression models were created using significant parameters detected in univariate survival analysis: age, ALDH1 positivity, CD44 positivity. According to Cox regression model, only the ALDH1 positivity was found to be a significant independent factor for DFS, with positivity associated significantly increasing the poor prognosis HR:16.4 (95% CI: 3.6-73.5, p = 0.0001).

Discussion

Laryngeal carcinoma (LC) is the second most prevalent head and neck cancer, with rising mortality rates in the United States [24]. According to several previous constructive clinical trials, surgery, radiotherapy, and chemotherapy are the effective treatment approaches of LC based on different tumor stages [24, 25, 26]. Identifying novel biomarkers is necessary for its diagnosis, treatment, and prognostic assessment. Several prognostic factors can be used for the evaluation of laryngeal cancer. Microscopic grade is an independent prognostic factor and correlates with clinical stage [12]. Diabetes, underweight, and conventional dose fractionation decrease the probability of curative effect of radiotherapy in elderly glottic cancer patients. A high number of comorbid diseases diminishes the probability of long-term survival in elderly glottic cancer patients [27]. For example, the overexpression of cycloxygenase-2 (COX-2), p53, epithelial cell adhesion molecule (EpCAM), hypoxia inducible factor 1α subunit, and carbonic anhydrase IX has been reported as a risk factor for a high local recurrence rate, and a high total microvessel perimeter per tumor area was a predictor of 337 optimized radiation therapy strategies for early GC [28, 29, 30, 31]. However, in a recent systematic review, EGFR and P53 could not predict LC after radiation therapy [31] and other clusters of markers (markers involved in angiogenesis and hypoxia, apoptosis markers, cell cycle markers, COX-2, and DNA properties) also did not provide evidence for the prediction of LC after radiation therapy. Another biomarker is Cdc42-interacting protein-4 (CIP4). In a study of Fung *et al*., the authors reported that CIP4 is a potential prognostic factor in patients with larynx carcinoma. The CIP4 expression level was downregulated with tumor progression [32]. Recently, a small population of cancer cells referred to as CSCs has been implicated in tumor initiation, relapse, and resistance to chemotherapy or RT; as a result, eradication of CSCs is considered essential in cancer therapy [15, 16]. The CSC hypothesis has also been coined for HNSCC in the head and neck; some cell surface markers have been reported as CSC markers in HNSCC cancers, such as CD44, CD133, ALDH1 and ABCG2 [17, 18, 19], and high expression of these markers is usually considered an indicator of poor prognosis. Among them, CD44 is the most reported CSC marker in HNSCC [20, 21, 22]. Chen *et al*. reported that ALDH1-positive cells are phenotypic and functional precursors of CSCs. These cells develop from ALDH1 cell lines and have more proliferative activity [33]. In the same study, it was found that ALDH1-positive cells had similar genetic structure to mesenchymal stem cells. Guided by these studies, ALDH1 expression is thought to be a good biomarker for CSCs in head and neck cancers [33, 34].

A study by Chen *et al*. showed that colony formation was stimulated with 0-10 Gy radiation in isolated ALDH1-positive cells. The study also compared CD44-positive, CD24-negative cells that were positive and negative for ALDH1. A total of 226 HNSCC patients who were positive for ALDH1 were evaluated, and ALDH1 levels were found to be associated with advanced-stage disease and undifferentiated tumors. In addition, it was determined that patients who were positive for ALDH1 had poorer survival with oncological treatment [35]. In an experimental study, the authors reported that a small portion of the population of CD 44 positive cancer cells, which typically comprise < 10% of the cells in a head and neck squamous cell carcinoma tumor, but not CD44 negative cancer cells, gave rise to new tumor *in vivo*. Immunohistochemistry showed that CD44 positive cancer cells have a primitive cellular structure and costain with the basal cell marker cytokeratin 5/14, whereas CD44 negative cancer cells resemble differentiated squamous epithelium and express the differentiation marker involucrin. The tumors that arose from purified CD44 positive cells reproduced the original tumor heterogeneity and could be serially passaged, thus demonstrating the two defining properties of stem cells: ability to self-renew and to differentiate [36].

In our study, the prevalence of ALDH1 positivity was significantly higher among patients with tumor recurrence due to RT failure. Al-Assar *et al*. reported that several biomarkers such as CD44, CD24, CD133, and epithelial-specific antigen may be related to radiosensitivity. In this study, it was found that CSCs, except those that were CD24-negative, did not have a radioresistant phenotype [37]. In a meta-analysis, ALDH1 expression was reported to be associated with low overall and disease-related survival [38]. In contrast, Lopez-Gonzales reported that patients with stage 1-2 lung cancer and ALDH1 nuclear expression showed good survival [39]. This seems completely opposite to our study, in which ALDH1 positivity was significantly more common among patients with failed RT while CD44 positivity was not significantly different between the two groups.

ALDH1 positivity was found to be an independent risk factor for DFS and significantly associated with poor prognosis.

There are some limitations for this study, e.g. The sample size is very small because patients with anterior commissure involvement were excluded from the study, and not specifying the smoking status and comorbidities of the patients.

Conclusions

The results of our study demonstrate that ALDH1 positivity was associated with disease-related survival and RT sensitivity. CD44 positivity did not differ between patients with and without recurrence after RT. In our study, the sample size was small and there need to be larger cohort studies to confirm the predictive effects of these markers. For ALDH1 positivity to be used as a biomarker when making treatment decisions, it is necessary to conduct studies with a larger patient series and determine its correlation with other biomarkers.

Key points:

- Immunohistochemical positivity for ALDH1 was found to be significant risk factor for RT failure in patients with laryngeal SCC.
- CD44 positivity and age group were not significantly associated with RT response.
- ALDH1 positivity was a significant predictor of disease-related survival and RT sensitivity.
- ALDH1 positivity may be a useful biomarker when determining choice of treatment.

We would like to thank Prof. Dr. Gulsah Seydaoglu for statistical analysis.

The authors declare no conflicts of interest.

References

1. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2012. CA. Canc J Clinician 2012; 62: 10-29.
2. Kokko LL, Hurme S, Maula SM, et al. Significance of site-specific prognosis of cancer stem cell marker CD44 in head and neck squamous cell carcinoma. Oral Oncol 2011; 47: 510-516.
3. Institute NC. Cancer Stat Facts: Larynx Cancer Surveillance, Epidemiology, and Results (SEER) database. 2016.
4. Wolf GT, Fisher SG, Hong WK, et al. Induction chemotherapy plus radiation comparedd with surgery plus radiation in patients with advanced laryngeal cancer. N Engl J Med 1991; 324: 1685-1690.
5. Ries LA, Harkins D, Krapcho M, et al. SEER cancer statistics review, 1975-2003. National Cancer Institute, Bethesda 2006.
6. Mendenhall WM, Amdur RJ, Morris CG, Hinerman RW. T1-T2N0 squamous cell carcinoma of the glottic larynx treated with radiation therapy. J Clin Oncol. 2001;19: 4029-4036.
7. Karatzanis AD, Psychogios G, Zenk J, et al. Comparison among different available surgical approaches in T1 glottic cancer. Laryngoscope 2009; 119: 1704-1708.
8. Silver CE, Beitler JJ, Shaha AR, Rinaldo A, Ferlito A. Current trends in initial management of laryngeal cancer: the declining use of open surgery. Eur Arch Otorhinolaryngol 2009; 266: 1333-1352.
9. Yoo J, Lacchetti C, Hammond JA, Gilbert RW; Head and Neck Cancer Disease Site Group. Role of endolaryngeal surgery (with or without laser) versus radiotherapy in the managementt of early (T1) glottic cancer: a systematic review. Head Neck 2014; 36: 1807-19.
10. Mendenhall WM, Werning JW, Hinerman RW, et al. Management of T1-T2 glottic carcinomas. Cancer 2004; 100: 1786-92.8.
11. Abdurehim Y, Hua Z, Yasin Y, et al. Transoral laser surgery versus radiotherapy: systematic review and meta-analysis for treatment options of T1a glottic cancer. Head Neck 2012; 34: 23-33.
12. Wiernik G, Millard PR, Haybittle JL. The predictive value of histological classification into degrees of differentiation of squamous cell carcinoma of the larynx and hypopharynx compared with the survival of patients. Histopathology 1991; 19: 411-417.
13. Westerbeek HA, Mooi WJ, Hilgers FJ, et al. Ploidy status and the response of T1 glottic carcinoma to radiotherapy. Clin Otolaryngol 1993; 18: 98-101.
14. Gallo O, Libonati GA, Gallina E, et al. Langerhans cells related to prognosis in patients with laryngeal carcinoma. Arch Otolaryngol Head Neck Surg 1991; 117: 1007-1010.
15. Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. Ann Rev Dev Biol 2007; 23: 675-699.
16. Bomken S, Fiser K, Heidenreich O, Vormoor J. Understanding the cancer stem cell. Br J Canc 2010; 103: 439-445.
17. Chiou SH, Yu CC, Huang CY, et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Canc Res: Offic J Am Assoc Canc Res 2008; 14: 4085-4095.
18. Yu CC, Chang YC. Enhancement of cancer stem-like and epithelial mesenchymal transdifferentiation property in oral epithelial cells with long-term nicotine exposure: reversal by targeting SNAIL. Toxicol Appl Pharmacol 2013; 266: 459-469.
19. Chen YS, Wu MJ, Huang CY, et al. CD133/Src axis mediates tumor initiating property and epithelial-mesenchymal transition of head and neck cancer. PloS One 2011; 6: e28053.
20. Trapasso S, Allegra E. Role of CD44 as a marker of cancer stem cells in head and neck cancer. Biol Targets Ther 2012; 6: 379-383.
21. Joshua B, Kaplan MJ, Doweck I, et al. Frequency of cells expressing CD44, a head and neck cancer stem cell marker: correlation with tumor aggressiveness. Head Neck 2012; 34: 42-49.
22. Chikamatsu K, Takahashi G, Sakakura K, et al. Immunoregulatory properties of CD44+ cancer stem-like cells in squamous cell carcinoma of the head and neck. Head Neck 2011; 33: 208-215.
23. Amin M, Edge S, Greene F, et al. AJCC cancer staging manual. 8th ed. Springer, New York 2017.
24. Baird BJ, Sung CK, Beadle BM, Divi V. Treatment of early-stage laryngeal cancer: a comparison of treatment options. Oral Oncol 2018; 87: 8-16.
25. Warner L, Chudasama J, Kelly CG, et al. Radiotherapy versus open surgery versus endolaryngeal surgery (with or without laser) for early laryngeal squamous cell cancer. Cochrane Database Syst Rev 2014; 2014: CD002027.
26. Obid R, Redlich M, Tomeh C. The treatment of laryngeal cancer. Oral Maxillofac Surg Clin North Am 2019; 31: 1-11.
27. Mucha-Małecka A, Małecki K, Amrogowicz N, et al. Prognostic factors in elderly patients with T1 glottic cancer treated with radiotherapy. Sci Rep 2021; 11: 17717.
28. Murakami N, Mori T, Yoshimoto S, et al. Expression of Ep-CAM and prognosis in early-stage glottic cancer treated by radiotherapy. Laryngoscope 2014; 124: 431-436.

29. Schrijvers ML, van der Laan BF, de Bock GH, et al. Overexpression of intrinsic hypoxia markers HIF1alpha and CA-IX predict for local recurrence in stage T1-T2 glottic laryngeal carcinoma treated with radiotherapy. Int J Radiat Oncol Biol Phys 2008; 72: 161-169.
30. Zhang S, Hayashi R, Fujii M, et al. Total microvessel perimeter per tumor area is a predictor of radiosensitivity in early-stage glottic carcinoma. Int J Radiat Oncol Biol Phys 2009; 73: 1104-1109.
31. Noordhuis MG, Kop EA, Bert van der Vegt, et al. Biological tumor markers associated with local control after primary radiotherapy in laryngeal cancer: a systematic review. Clin Otolaryngol 2020; 45: 486-494.
32. Fang L, Shi L, Wang W, et al. Low expression of CIP4 in predicting worse overall survival: a potential biomarker for laryngeal cancer. PLoS One 2021; 16: e0253545.
33. Chen C, Wei Y, Hummel M, et al. Evidence for epithelial-mesenchymal transition in cancer stem cells of head and neck squamous cell carcinoma. PLoS One 2011; 6: e16466.
34. Greco N, Schott T, Mu X, et al. ALDH activity correlates with metastatic potential in primary sarcomas of bone. J Cancer Ther 2014; 5: 331-338.
35. Chen YC, Chen YW, Hsu HS, et al. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophys Res Commun 2009; 385: 307-313.
36. Prince ME, Sivanandan R, Kaczorowski A, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S a 2007; 104: 97973-97978.
37. Al-Assar O, Muschel RJ, Mantoni TS, et al. Radiation response of cancer stem-like cells from established human cell lines after sorting for surface markers. Int J Radiat Oncol Biol Phys 2009; 75: 1216-1225.
38. Zhou C, Sun B. The prognostic role of the cancer stem cell marker aldehyde dehydrogenase 1 in head and neck squamous cell carcinomas: a meta-analysis. Oral Oncol 2014; 50: 1144-1148.
39. Lopez-Gonzalez A, Salas C, Provencio M, et al. Aldehyde dehydrogenases in early stage lung cancer: nuclear expression. Clin Transl Oncol 2014; 16: 931-934.

Address for correspondence

Dr. Ozgur Surmelioglu
Department of Otorhinolaryngology
Faculty of Medicine
Cukurova University
Adana, Turkey
e-mail: surmeli2004@yahoo.com