

Claudin-1 in Head and Neck Squamous Cell Carcinoma

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Keywords

Head and neck cancer · Head and neck squamous cell carcinoma · Claudin-1 · Gene expression · Prognostic factor

Abstract

Introduction: The objective response rate to immunotherapy is limited in recurrent/metastatic head and neck squamous cell carcinoma (HNSCC) patients, whose prognosis is still dismal. Few prognostic factors are clinically available, mostly related to patient or disease characteristics. Gene expression signatures offer better prognostic abilities but are mainly used in research. One such GE model classifies HNSCC into 6 clusters with different prognoses. Claudin-1 (CLDN1), which influences tumor microenvironment and immune cell infiltration, has emerged as a potential target, especially in cancers like HNSCC with high CLDN1 expression. **Methods:** A single-center cohort of 100 loco-regionally advanced HNSCC patients from the BD2Decide observational study was analyzed. Patients were selected to balance long-term survivors and deceased patients, including HPV-negative and HPV-positive cases. Primary tumor specimens underwent GE analysis using Affymetrix ClariomD chips. Primary endpoint was overall survival (OS). **Results:** The cohort comprised 100 HNSCC patients with a median age of 60 years, predominantly men (76%). Median OS and disease-free survival (DFS) were 94.24

and 42.79 months, respectively. CLDN1 expression varied significantly among primary sites, being highest in hypopharynx cancers. Differences in expression were not significant when stratified by HPV status or clinical stage. CLDN1 expression differed across the 6 transcriptomic clusters, with the highest levels in clusters associated with mesenchymal and hypoxic features. Higher CLDN1 expression correlated with shorter OS (hazard ratio [HR]: 3, $p = 0.0023$) and DFS (HR: 2.14, $p = 0.02$). **Conclusion:** CLDN1 expression is heterogeneous in HNSCC and carries prognostic significance. It is highest in tumors with HPV-like biology and hypoxic environments, and lowest in immune-sensitive clusters. High CLDN1 is a negative prognostic factor and a promising therapeutic target. Anti-CLDN1 treatments could improve outcomes of CLDN1+ HNSCC patients, and combination therapies with ICIs might overcome resistance in CLDN1– cases. These findings support the need for clinical studies on anti-CLDN1 therapies.

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Introduction

Anti-PD1 immune checkpoint inhibitors (ICIs), with or without chemotherapy, are the standard of care for recurrent/metastatic (R/M) head and neck squamous cell

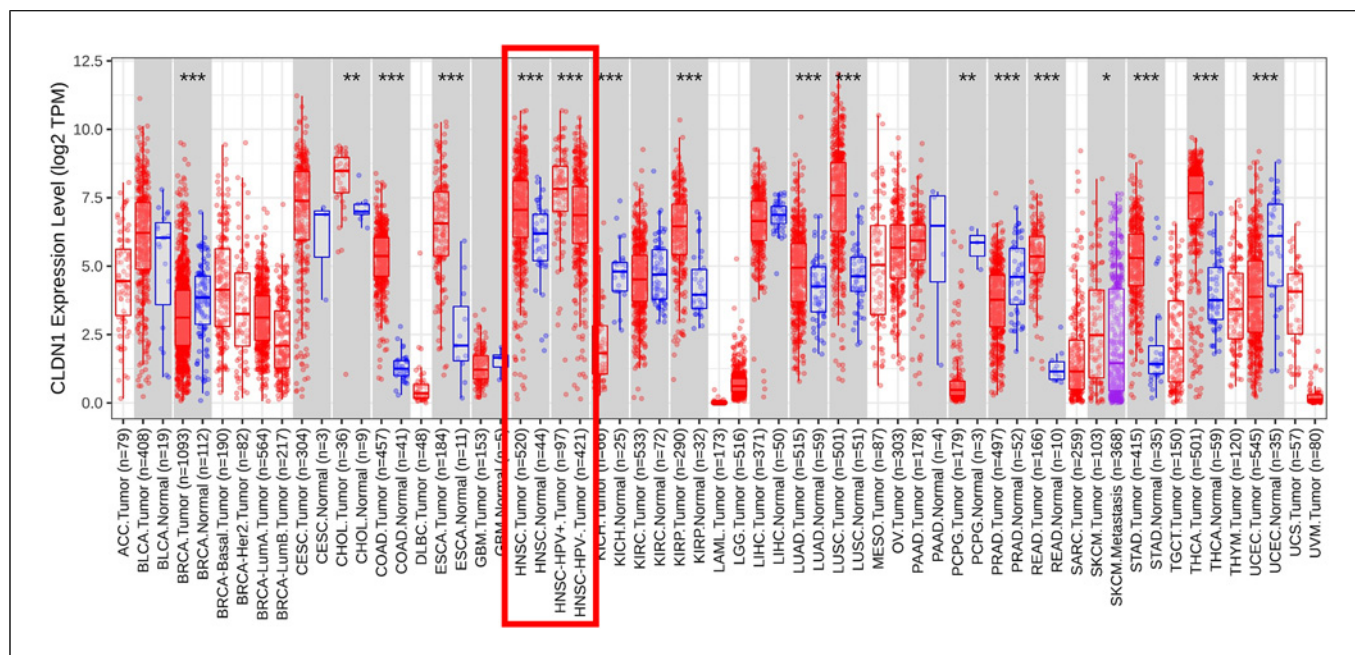


Fig. 1. CLDN1 expression level (log2) in the TCGA.

carcinoma (HNSCC) patients [1]. However, HNSCC is known to be related to immune suppression [2]. Indeed, the objective response rate to ICI is limited, and patient prognosis is still unfavorable.

Only a few prognostic factors are available in clinical practice, and they are limited to patient or disease characteristics (e.g., performance status, comorbidities, HPV status, primary tumor site, stage). Gene expression (GE) signatures have shown better prognostic ability, but their use is still limited to research. Among validated GE models, one signature classifies HNSCCs into six clusters, with different biological features and prognosis [3].

In addition to better patient selection and survival forecasting, there is an urgent need to improve oncologic outcomes. Several strategies to enhance immunotherapy activity have failed, including ICI combinations [3] and association with antiangiogenic agents [4]. Acting on tumor microenvironment to overcome immune suppression is one of the possible ways to enrich the therapeutic options [2].

Claudin-1 (CLDN1), an emerging actionable target, leads to myoepithelial transformation and fibrogenesis in cancer microenvironment. The abundance of non-junctional CLDN1 promotes collagen alignment and tumor-stroma interface remodeling, hampering immune cell infiltration [5].

CLDN1 overexpression can be found in fibrotic diseases (e.g., liver fibrosis and cirrhosis) and cancer [6], with variable expression across solid tumors. HNSCC has the highest CLDN1 expression (TCGA data, Fig. 1). The present work aimed to explore CLDN1 GE in a cohort of HNSCC patients.

Methods

A single-center cohort of loco-regionally advanced HNSCC patients treated with curative intent and included in the BD2Decide observational study was retrospectively analyzed [7]. Patients were diagnosed between 2008 and 2017 (end of the follow-up period: June 2019). Given the retrospective nature of the analysis, no formal sample size calculation was performed. We applied the following criteria to ensure a numerical balance between long-term survivors and deceased patients: (i) for each of the primary HPV-negative cancer sites (oral cavity, HPV-negative oropharynx, hypopharynx, and larynx cancers), we selected 20 patients, 10 died of disease and 10 alive and disease-free with at least 2 years of follow-up; (ii) given the better prognosis of HPV-related oropharynx cancer patients, we selected 20 subjects, 10 experiencing disease recurrence and 10 alive and disease-free with at least 2 years of follow-up.

The following clinical data were collected: age (continuous variable), sex, HNSCC site and HPV status (oral cavity, HPV-negative oropharynx, HPV-positive oropharynx, hypopharynx, larynx), clinical stage (AJCC/UICC eighth edition), GE cluster according to De Cecco et al. [8]. Patients with missing data were excluded.

Primary tumor specimens were formalin-fixed paraffin-embedded samples. After macrodissection to obtain $\geq 70\%$ of tumor cells without significant necrosis (details on the protocol of GE analysis have been previously described [7]; assays were performed blinded to the study endpoint, i.e., survival), specimens were analyzed for GE by Affymetrix ClariomD chips and processed using the Transcriptome Analysis Console Software (Thermo Fisher). Normalized and log₂ CLDN1 were retrieved from the data matrix. The distribution of CLDN1 expression was assessed based on anatomical sites, HPV status, and the six GE clusters (Cl) by De Cecco et al. [8]. The distributions among groups were compared using Mann-Whitney and Kruskal-Wallis tests, as appropriate.

Median follow-up was estimated using the reverse Kaplan-Meier method. Overall survival (OS) was the primary endpoint, and disease-free survival (DFS) was secondary. Multivariable models were performed comparing CLDN1 and clinical stage (AJCC/UICC seventh edition [TNM7]). Although this version was older than the eighth edition, it was the most recent staging system independent of HPV status and the current gold standard for prognostic forecasting in oncology. Hazard ratios (HRs) were calculated using the Cox proportional hazard model. In all prognostic analyses (i.e., univariate and multivariable), CLDN1 was handled as a continuous variable, and no cutoff was determined due to the limited sample size.

Statistical significance was set at 0.05. Statistical analyses were performed using R v4.1.0 Statistical Software, survival R package, and SAS[®] OnDemand for Academics. The BD2Decide observational study was approved by the Ethical Committee of the Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy) in 2016 (INT65-16, INT66-16).

Results

Based on the criteria specified in the Methods section, a cohort of 100 HNSCC patients was selected, including 20 cases for each of the five primary HNSCC sites. The median age was 60 (range 21–80), and 76% of subjects were men. In HPV-negative HNSCC patients, the clinical

stage was III in 25 cases (31%) and IVa/b in the remaining 55 (69%). In the HPV-positive oropharyngeal cancer cohort, the stage was I in 6 cases (30%), II in 6 (30%), and III in 8 cases (40%).

After a median follow-up of 64.44 months (95% CI: 54.24–66.91), median OS and DFS were 94.24 months (95% CI: 59.08–94.24) and 42.79 months (95% CI: 22.5–72.2), respectively. In the HPV-related oropharyngeal cancer cohort, the median OS was not reached, and the median DFS was 53.91 months (95% CI: 13.09–53.91). In HPV-negative HNSCC patients, median OS and DFS were 72.2 months (95% CI: 43.88–94.24) and 35.53 months (95% CI: 15.43–72.2), respectively.

CLDN1 expression significantly differed among HNSCC primary sites ($p = 0.0158$, Fig. 2a). In particular, the site with the highest median expression was hypopharynx, followed by HPV+ oropharynx, larynx, HPV– oropharynx, and oral cavity. No statistical significance was found stratifying cases according to HPV status ($p = 0.2215$, Fig. 2b) or clinical stage ($p = 0.4998$ in HPV-negative disease, $p = 0.9054$ in HPV-positive oropharynx).

The distribution of CLDN1 significantly differed across the six transcriptomic clusters ($p < 2.92 \times 10^{-6}$, Fig. 2c). The highest expressions were observed in Cl1 (HPV-like), Cl2 (mesenchymal), and Cl3 (hypoxia), the lowest in Cl4 (defense response) and Cl6 (immune-reactive) and Cl5 (classical) had an intermediate expression. Higher CLDN1 expression was associated with shorter OS (HR: 3, 95% CI: 1.43–6.28, $p = 0.0023$; Kaplan-Meier curve in online suppl. material; for all online suppl. material, see <https://doi.org/10.1159/000540775>), and DFS (HR: 2.14, 95% CI: 1.11–4.11, $p = 0.02$). At bivariate analysis, CLDN1 maintained a statistical significance in OS (HR: 1.22, 95% CI: 1.01–1.49, $p = 0.04$), not in DFS (HR: 1.05, 95% CI: 0.88–1.24, $p = 0.572$), while TNM7 was not significantly in both OS (HR: 1.56, 95% CI: 0.79–3.06, $p = 0.195$), and DFS (HR: 0.84, 95% CI: 0.44–1.57, $p = 0.591$).

Discussion

Our analyses showed that CLDN1 expression is heterogeneous in HNSCCs and has a prognostic significance. CLDN1 expression differs across primary tumor sites and GE clusters. Among the latter, the highest expression was observed in tumors with an HPV-like biology (Cl1) and those with the most hypoxic and immune-excluded microenvironment (Cl2-mesenchymal, Cl3-hypoxia). Transcriptomic features associated with sensitivity to

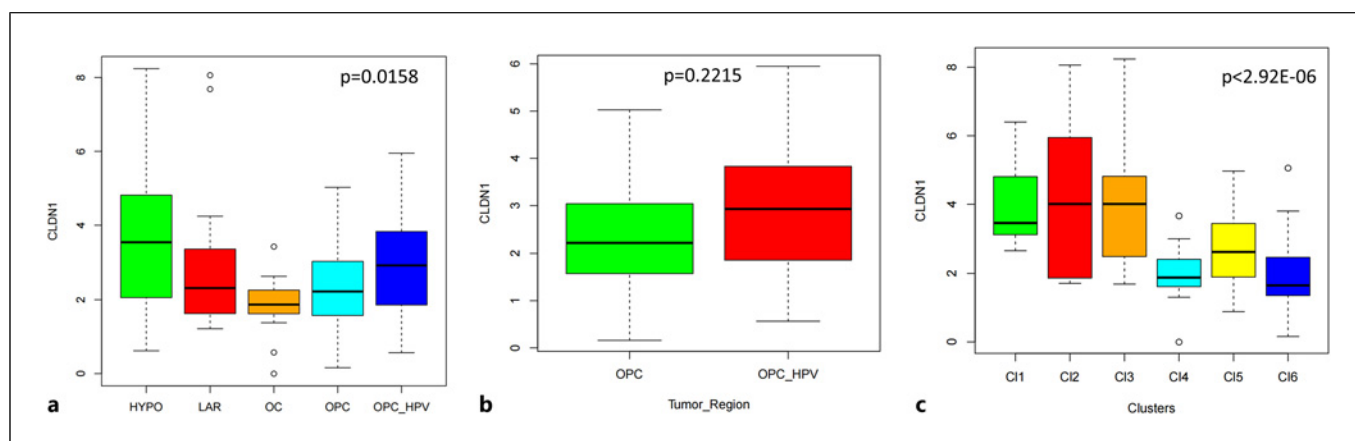


Fig. 2. CLDN1 expression based on primary HNSCC site (a), HPV status (b), and gene expression signature (c).

ICIs, notably Cl6 (immune-reactive) [9], had the lowest CLDN1 expression. Consistently, the association of CLDN1 expression with GE signatures characterized by epithelial-mesenchymal transition and fibrotic features is consistent with the literature [10].

Higher CLDN1 levels were associated with a significantly shorter DFS and OS, hence confirming the unfavorable prognosis of patients with a CLDN1-enriched disease [10]. Given the better prognosis of HPV-related oropharyngeal cancer patients, it is likely that CLDN1 expression may have a different role in virus-related and unrelated tumors.

Our study offers significant novelty by comprehensively analyzing CLDN1 expression in HNSCC patients, correlating its expression with GE clusters, and evaluating its prognostic significance in OS and DFS. Unlike previous studies, which primarily focused on the overexpression and prognostic roles of claudins in HNSCC and oral squamous cell carcinoma, respectively [11, 12], our research uniquely integrates CLDN1 expression with transcriptomic clusters characterized by different biological features and prognoses. Moreover, our study identifies CLDN1 as a potential therapeutic target, particularly in clusters associated with mesenchymal and hypoxic features, which were not previously highlighted in the same context.

Our findings align with and extend the results from prior research. Nelhübel et al. [11] demonstrated increased CLDN1 expression in HNSCC compared to normal epithelia and suggested its potential role as a therapeutic target. However, they did not delve into its relationship with distinct GE clusters or its implications in targeted therapy with ICIs. Dos Reis et al. [12] highlighted CLDN1's role in enhancing invasiveness and its association with aggressive histological features in oral cavity cancer but did not explore its differential ex-

pression in HNSCC GE subtypes or its potential as a marker for therapeutic interventions.

Limitations of this work include its single-center, retrospective design and relatively small sample size, which may limit generalizability and introduce potential biases. We did not include functional assays to elucidate the mechanistic role of CLDN1 or explore interactions with other claudins, and no immunohistochemical correlate was provided.

Nonetheless, these results support CLDN1 as a target in R/M HNSCC. Indeed, CLDN1+ HNSCC patients may benefit from anti-CLDN1 treatment. At the same time, in CLDN1- HNSCC patients, the association of an anti-PD1 drug with anti-CLDN1 agents may be a promising strategy to tackle both primary and secondary ICI resistance.

These results may guide patient selection for future clinical studies with anti-CLDN1 antibodies, including anti-CLDN1 antibody-drug conjugates. A clinical study of ALE.C04 (anti-CLDN1 antibody) is ongoing in pre-treated R/M HNSCC patients (ClinicalTrials.gov identifier: NCT06054477).

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Statement of Ethics

This study protocol was reviewed and approved by the Ethical Committee of the Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, via Giacomo Venezian 1, 20133 Milan, Italy, Approval No. INT65-16, INT66-16. All patients from the BD2Decide observational study provided written informed consent.

Conflict of Interest Statement

Lisa Licitra declares research funds to the institute for clinical studies from AstraZeneca, BMS, Boehringer Ingelheim, Celgene International, Eisai, Exelixis, Debiopharm International SA, Hoffmann-La Roche Ltd., IRX Therapeutics, Medpace, Merck-Serono, MSD, Novartis, Pfizer, Roche, Buran, and Alentis and occasional fees for participation as a speaker at conferences/congresses or as a scientific consultant for advisory boards from AstraZeneca, Bayer, MSD, Merck-Serono, AccMed, and Neutron Therapeutics, Inc. Stefano Cavalieri declares occasional fees for participation as a speaker at conferences/congresses from AccMed and support for attending meetings and/or travel from AccMed, MultiMed Engineers srl, and Care Insight SAS. The remaining authors have no conflicts of interest to declare.

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Author Contributions

S.C.: data curation, formal analysis, investigation, methodology, visualization, writing – original draft, and writing – review and editing. C.B.: data curation, project administration, and writing – review and editing. D.L., M.L., and E.T.: formal analysis, methodology, resources, and writing – review and editing. A.O.: data curation, project administration, and writing – review and editing. L.L.: conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, and writing – review and editing. L.D.C.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, writing – original draft, and writing – review and editing.

Data Availability Statement

The data that support the findings of this study are not publicly available because their containing information that could compromise the privacy of research participants but are available from the corresponding author (S.C.) upon reasonable request.

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