Identification of claudin-1, -3, -7 and -8 as prognostic markers in human laryngeal carcinoma

SHU ZHOU[1], XUE PIAO[2], CHENGYAN WANG[3], RUI WANG[3] and ZHIMIN SONG[4]

[1]Department of Anesthesiology, Jilin Cancer Hospital; [2]Department of Anesthesiology, Maternity Hospital of Changchun City; [3]Department of Ultrasound, Jilin Cancer Hospital; [4]Department of Anesthesiology, The Second Hospital of Jilin University, Changchun, Jilin 130021, P.R. China

Received June 20, 2018; Accepted February 8, 2019

DOI: 10.3892/mmr.2019.10265

Abstract. Various genomic and epigenetic modifications that occur during the development of cancer act as potential biomarkers for early diagnosis and treatment. Previous studies have demonstrated abnormal expression of the claudin (CLDN) tight junction (TJ) proteins in numerous types of human cancer. Reverse transcription-quantitative polymerase chain reaction and western blotting were employed to investigate variations in the expression of the CLDN TJ proteins in laryngeal non-neoplastic tissues and laryngeal squamous carcinoma tissues. It was revealed that CLDN2, CLDN4, CLDN5, CLDN6, CLDN9, CLDN11 and CLDN12 were undetectable in laryngeal squamous carcinoma tissues and laryngeal non-neoplastic tissues. Additionally, CLDN10 was expressed in laryngeal squamous carcinoma tissues and laryngeal non-neoplastic tissues; however, no significant difference was reported. Conversely, the expression levels of CLDN1 and CLDN7 mRNA and protein were downregulated in laryngeal squamous carcinoma tissues compared with in adjacent non-neoplastic tissues, whereas those of CLDN3 and CLDN8 were upregulated. A total of 80 samples of laryngeal squamous carcinoma and non-neoplastic tissues were analyzed for the expression of CLDN1, -3, -7 and -8 via streptavidin-peroxidase immunohistochemical staining. It was revealed that the expression levels of CLDN1 and CLDN7 were downregulated in laryngeal squamous carcinoma tissues compared with in non-neoplastic mucosal tissues, whereas those of CLDN3 and CLDN8 were upregulated. Furthermore, the associations between CLDN expression and the clinicopathological factors of patients were analyzed. The expression levels of CLDN3 and CLDN7 were reported to be associated with distant metastasis and serve as potential predictors of poor prognosis. In conclusion, the findings of the present study demonstrated that the expression levels of CLDN1, -3, -7 and -8 varied between laryngeal squamous carcinoma tissues and non-neoplastic tissues. The expression levels of these CLDNs may be useful molecular markers for the diagnosis of laryngeal carcinoma, and determining the metastasis and prognosis of this disease.

Correspondence to: Dr Zhimin Song, Department of Anesthesiology, The Second Hospital of Jilin University, 218 Ziqiang Street, Changchun, Jilin 130021, P.R. China
E-mail: szmjlu@126.com

Key words: tight junction, claudin-1, claudin-3, claudin-7, claudin-8, laryngeal carcinoma

Introduction

Tight junctions (TJs) form the apical junctional complex in epithelial and endothelial cellular sheets in cooperation with adherens junctions and desmosomes (1). TJs are important for the tight structure of cellular sheets, which enables monitored paracellular ion flux and the maintenance of tissue homeostasis (2). At present, >40 diverse proteins have been identified in the TJs of epithelia and endothelia (3,4). TJs exhibit a cement-like function and prevent the detachment of epithelial cells (5). An important step during the initiation of cancer metastasis is contact with and dissemination of the vascular endothelium by disconnected tumor cells (6); therefore, TJs are the first obstacle that tumor cells are required to overcome to metastasize (7). TJs comprise three major types of fundamental membrane protein: Occludin, claudins (CLDNs) and junctional adhesion molecules (8,9). The precise characteristics of these proteins remain unclear; however, improved understanding of the molecular construction of TJs led to the development of models that identified TJs as present in diverse tissues and responsive to fluctuating natural, pathological or experimental surroundings (10,11).

The CLDN family of transmembrane proteins serves important roles in the formation of TJs and comprises ~27 members, the majority of which bind with PDZ domain-containing proteins (12,13). The theory of TJs as a purely paracellular barrier has been altered to consider its involvement in signaling cascades that regulate the proliferation and differentiation of cells (14). Thus, CLDNs are associated with multimolecular complexes and the transduction of cell signaling pathways (15-17). CLDNs have been reported to be associated with the regulation of proliferation, differentiation and other cellular functions via interactions with signaling proteins (18,19). The expression profile of CLDNs is tissue-specific; however, the majority of tissues express various CLDNs, which can recruit homotypic and heterotypic CLDNs for the formation of TJs (20). The combination of CLDNs comprising TJs determine their selectivity and

strength (21). CLDNs assemble as polymers inside cells that cooperate with the CLDNs of adjacent cells to form adhesive structures (22). The expression levels of CLDNs have been reported as altered in numerous types of tumor (23). Tumor cells commonly exhibit uncharacteristic CLDN expression profiles, in addition to reduced differentiation and cell polarity (24,25). CLDN1 has been reported to be downregulated in breast cancer and colon cancer (26); CLDN2 expression is also reduced in invasive breast cancer (27,28). Studies demonstrating decreased TJ protein expression in various types of cancer are consistent with the hypothesis that tumorigenesis is associated with the interruption of TJs, which may contribute to the damaged interconnectivity and suppressed differentiation of tumor cells.

Conversely, it has been reported that the expression levels of certain CLDNs are increased in tumor cells, suggesting that these proteins promote tumorigenesis (29,30). For example, studies employing serial analyses of gene expression have identified the expression of CLDN3 and CLDN4 as increased in ovarian cancer (31,32). The roles of CLDNs in cancer may be tissue-specific and depend on the precise molecular circuitry of the cell. In summary, abnormality of the CLDNs has been accepted as a factor that endows transformed epithelial cells with metastatic capability (22). However, the expression profiles of the CLDNs in laryngeal squamous carcinoma have yet to be determined (33). Thus, the aims of the present study were to investigate the expression of CLDNs in adjacent non-neoplastic laryngeal tissues and laryngeal squamous carcinoma tissues, and to determine associations between alterations of CLDNs and the clinicopathological characteristics of patients with laryngeal squamous carcinoma.

Materials and methods

Patients. Biopsies were obtained from 80 patients with a pathologically confirmed diagnosis of laryngeal squamous carcinoma who received treatment at Jilin Cancer Hospital (Changchun, China) between June 2007 and May 2012. The patients were selected based upon the following criteria: No history of radiotherapy and chemotherapy, and no prior malignant disease. The grade and classification of the laryngeal squamous patients were determined according to the American Joint Committee on Cancer tumor-node-metastasis staging system (34). Histologically verified non-neoplastic laryngeal tissues were collected >3 cm from the tumors. The patients included in the study comprised 46 males and 34 females and were between 32 and 76 years old, with an average age of 52 years. The medical records of the patients, including Ki67 expression, were reviewed to determine the clinical and pathological characteristics.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RT-qPCR was used to investigate the expression of CLDNs in laryngeal squamous tissues and adjacent non-neoplastic tissues from 6 patients. Total RNA was extracted using a RNAiso Plus (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocols. qPCR was conducted as previously described (35). The cDNA reaction products of RT were subjected to qPCR using a CTFX 96 Real-time system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and SYBR® Green Supermix (Bio-Rad Laboratories, Inc.) according to the manufacturer's protocol. The thermocycling conditions were: 95˚C for 30 sec, followed by 30 cycles of 95˚C for 10 sec, 60˚C for 32 sec, 95˚C for 15 sec, 60˚C for 60 sec and 95˚C for 15 sec. The following primer pairs were used for qPCR: CLDN1, forward 5'-GCCACAGCAAGGTATGGTAAC-3', reverse, 5'-AGTAGG GCACCTCCCAGAAG-3'; CLDN2, forward 5'-TTCATC GGCAACAGCATCG-3', reverse, 5'-GGTTATAGAAGTCCC GGATGA-3'; CLDN3, forward 5'-AGTGCAAGGTGTACGA CTC-3', reverse, 5'-AGTCCCGGATAATGGTGTTG-3'; CLDN4, forward 5'-TTGTCACCTCGCAGACCATC-3', reverse, 5'-GCAGCGAGTCGTACACCTTG-3'; CLDN5, forward 5'-AACATCGTGACGGCGCAGACCA-3', reverse, 5'-TCAGAGCCAGCACCGAGTCGTACA-3'; CLDN6, forward 5'-GGCAACAGCATCGTCGTGG-3', reverse, 5'-GAAGTC CTGGATGATAGAGTGGGC-3'; CLDN7, forward 5'-TTTTCA TCGTGGCAGGTCTT-3', reverse, 5'-GGCCAAACTCATACT TAATGTTGG-3'; CLDN8, forward 5'-TCTGCAGTAGGACAT AGAAACCCCTAA-3', reverse, 5'-CGTTTAGGGGTTTCT ATGTCCTACTGC-3'; CLDN9, forward 5'-CTAGCACTAGTT TCGAAATGGCTTCGACCGGCTTAG-3', reverse, 5'-TCT CGAGCTAGTCGACTCACACGTAGTCCCTCTTGTC-3'; CLDN10, forward 5'-GGAGGCTCCGATAAAGCCAA-3', reverse, 5'-GTGGCCCCGTTGTATGTGTA-3'; CLDN11, forward 5'-TGACCTGCAGCTACACCATC-3', reverse, 5'-GGG GTTTGCAGTGGTAGAGA-3'; CLDN12, forward 5'-CCG TGATGTCCTTCTTGGCTTTC-3', reverse, 5'-CTCTGATGA TGGCATTGGCAACC-3'; and GAPDH, forward 5'-AACGTG TCAGTCGTGGACCTG-3' and reverse, 5'-AGTGGGTGT CGCTGTFGAAGT-3'. Relative levels of mRNA expression were quantified using the $2^{-\Delta\Delta Cq}$ method and were normalized to GAPDH (30).

Western blotting. Western blotting was performed to detect the expression of CLDNs in laryngeal squamous tissues and adjacent non-neoplastic tissues from 6 patients. Total protein was extracted from laryngeal tissues using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) containing phenylmethylsulfonyl fluoride (Beyotime Biotechnology) and proteinase inhibitor cocktail solution (Roche Diagnostics, Basel, Switzerland). The total protein was washed with ice-cold PBS three times, and cell lysates were prepared with a lysis buffer containing 10 mM Tris-HCl (pH 7.4), 1% SDS and 1 mM Na_3VO_4. A Bicinchoninic Acid Protein Assay Kit (Pierce; Thermo Fisher Scientific, Inc.) was used to determine protein concentration. Total protein (30 μg) was separated via 10% SDS-PAGE and then transferred to a nitrocellulose membrane. Membranes were blocked with 5% fat-free dried milk at room temperature for 1 h and then incubated with the following primary antibodies (all 1:1,000 dilution) for 1 h at room temperature: Rabbit anti-human CLDN1 (sc-81796, Santa Cruz Biotechnology, Inc., Dallas, TX, USA); rabbit anti-human CLDN3 (sc-517546, Santa Cruz Biotechnology, Inc.); rabbit anti-human CLDN7 (ab27487, Abcam, Cambridge, UK); rabbit anti-human CLDN8 (ab183738, Abcam) and mouse anti-human β-actin (ab8226, Abcam). The membranes were subsequently washed three times with PBS and incubated with horseradish peroxidase-conjugated secondary antibodies (sc-2537; 1:1,000; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Immunoreactive bands were visualized using enhanced chemiluminescence western blotting reagents

(GE Healthcare, Chicago, IL, USA) and quantified using Image Lab 6.0.1 software (Bio-Rad Laboratories, Inc.).

Immunohistochemistry. Immunohistochemistry was performed to investigate the expression profiles of CLDNs in laryngeal squamous tissues and adjacent non-neoplastic tissues. Experiments were conducted as previously described (36), briefly, the immunohistochemical analysis was performed according to the manufacturer's protocols of UltraSensitive™ SP (Mouse/Rabbit) IHC Kit (cat. no. KIT-9710, Maixin Biological Technology Development Company, Fujian, China). The tissue was fixed overnight with 10% formaldehyde and embedded in paraffin wax. Following deparaffining, rehydration in a graded ethanol series and antigen retrieval with Citrate Buffer pH6.0 (1:300 dilution; ZLI-9065, OriGene Technologies, Inc., Beijing, China), paraffin sections (1.5 mm thick) were incubated at 4˚C overnight with the following antibodies: Rabbit anti-human CLDN1 antibody (1:300), rabbit anti-human CLDN3 antibody (1:400), rabbit anti-human CLDN7 antibody (1:400) and rabbit anti-human CLDN8 antibody (1:300). The levels of protein expression were determined based upon the percentage of positively stained tumor cells combined with the staining intensity as previously described (37). Subsequently, the slides were incubated with goat anti-rabbit amplification reagent (included in the IHC kit) for 30 min at room temperature and followed by incubation with diaminobenzidine (DAB) for 5 min at room temperature and counterstaining with hematoxylin. For negative controls, the tissue sections were incubated with isotype antibodies (diluted at same concentration with primary antibodies) the at 4˚C overnight. All sections were scored by two pathologists using a light microscope (E100; Nikon Corporation, Tokyo, Japan; magnification, x400).

Follow-up. Patients with a pathologically confirmed diagnosis of laryngeal carcinoma were followed-up from the beginning of diagnosis to 60 months for the analysis of occurrence, metastasis and survival. The mortal status of patients was obtained via a telephone interview or on an outpatient basis prior July 2017.

Statistical analysis. All experiments were repeated three times, and all data were presented as the mean ± standard deviation of at least three experimental results. Origin 7.5 laboratory data analysis software (OriginLab, Northampton, MA, USA) and image processing software (Image-Pro Plus 6.0, Media Cybernetics, Inc., Rockville, MD, USA) were used to quantify the data. The results were analyzed by a paired Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference. The χ^2/χ^2 goodness-of-fit tests were applied for the analysis of associations with clinicopathological indicators. In addition, the cohort was separated into tumors that were positive for CLDNs and those negative for CLDNs, and associations between CLDNs expression and clinical survival were analyzed via the Kaplan-Meier method and compared using log-rank tests.

Results

Expression levels of CLDN family members in laryngeal squamous tissues and adjacent non-neoplastic tissues. RT-qPCR and western blotting were performed to analyze the expression of CLDN family members in laryngeal squamous tissues and adjacent non-neoplastic tissues. As presented in Fig. 1A, the expression of CLDN2, CLDN4, CLDN5, CLDN6, CLDN9, CLDN11 or CLDN12 was not detected at the mRNA in the samples of laryngeal squamous carcinoma or adjacent non-neoplastic tissues. CLDN10 mRNA was expressed in laryngeal squamous carcinoma and non-neoplastic tissues; however, there was no significant difference was observed. Conversely, the levels of CLDN1 and CLDN7 expression were significantly downregulated at the mRNA and protein levels in

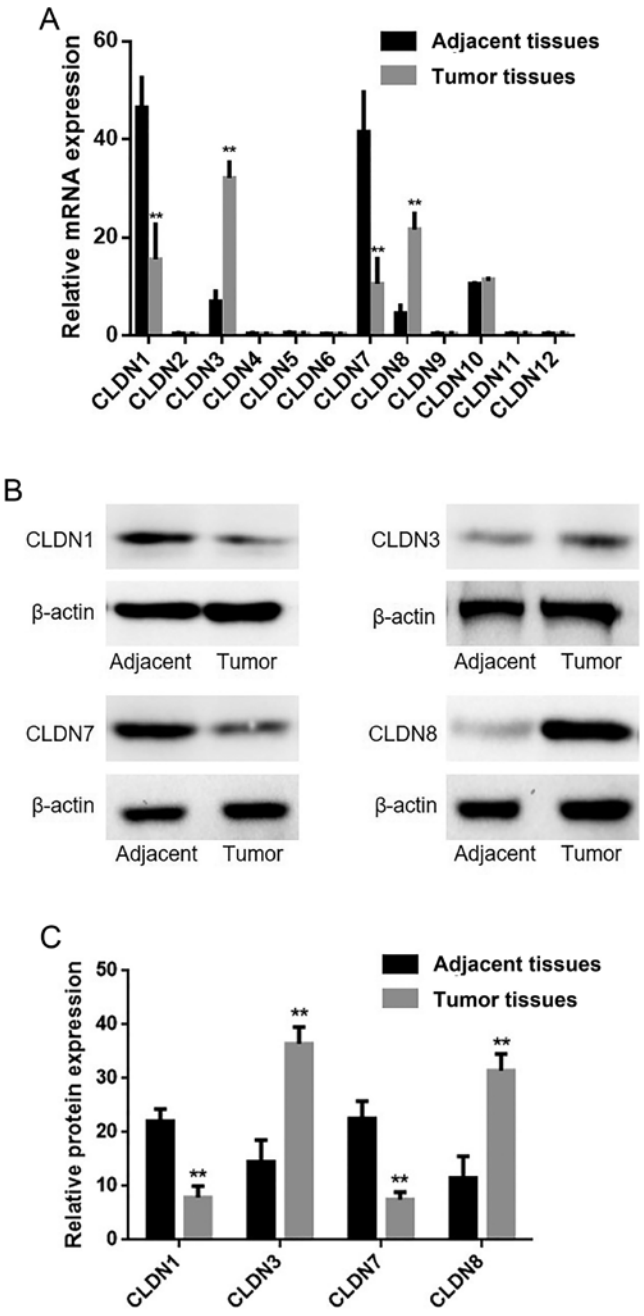

Figure 1. Expression of CLDNs in human laryngeal squamous carcinoma and adjacent non-neoplastic tissues. (A) Reverse transcription-quantitative polymerase chain reaction analysis of CLDN mRNA expression in laryngeal squamous carcinoma and adjacent non-neoplastic tissues. (B) Western blotting was used to investigate significant differences in the expression of CLDNs in laryngeal squamous carcinoma and adjacent non-neoplastic tissues. (C) Quantification of CLDN protein expression. Data are presented as the mean ± standard deviation. **P<0.01 vs. non-neoplastic tissues. CLDN, claudin.

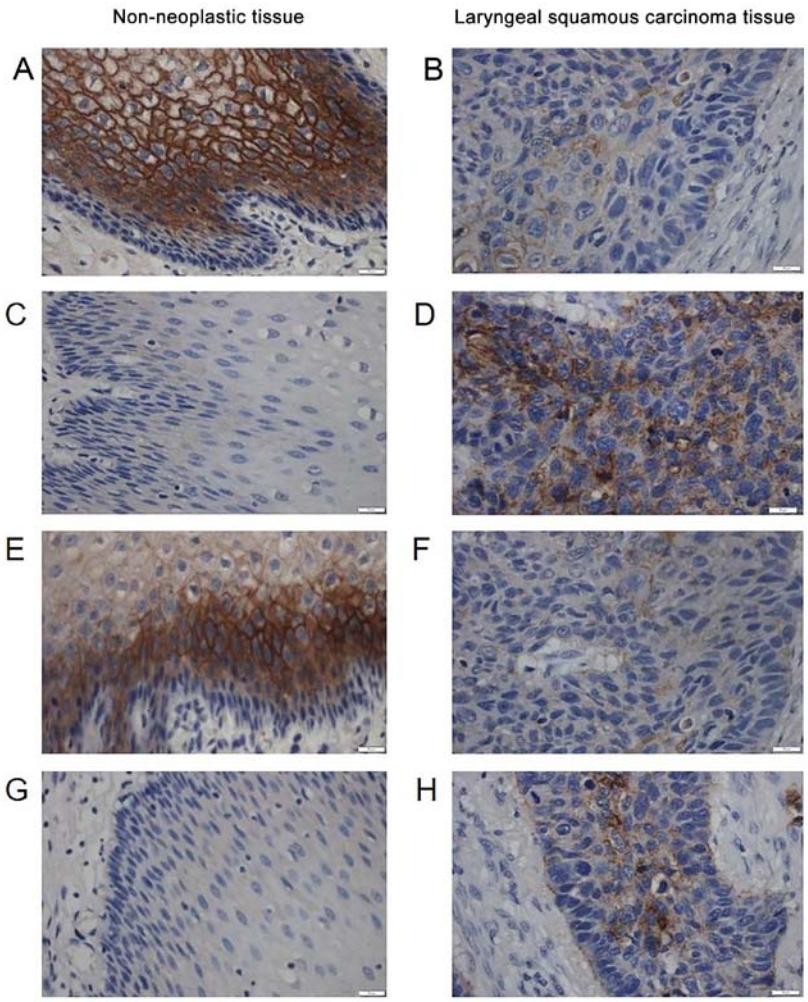

Figure 2. Immunohistochemical analysis of CLDN expression in human laryngeal squamous carcinoma and adjacent non-neoplastic tissues. High expression of CLDN1 in (A) non-neoplastic tissue compared with (B) laryngeal squamous carcinoma tissue. Low expression of CLDN3 in (C) non-neoplastic tissue compared with (D) laryngeal squamous carcinoma tissue. High expression of CLDN7 in (E) non-neoplastic tissue compared with (F) laryngeal squamous carcinoma tissue. Low expression of CLDN8 in (G) non-neoplastic tissue compared with (H) laryngeal squamous carcinoma tissue. Magnification, x400. CLDN, claudin.

laryngeal squamous carcinoma tissues compared with adjacent non-neoplastic tissues, whereas those of CLDN3 and CLDN8 were significantly upregulated (Fig. 1A-C).

CLDN1 and CLDN7 are downregulated, while CLDN3 and CLDN8 are upregulated in laryngeal squamous carcinoma. CLDN1 expression was evaluated in 80 pairs of laryngeal squamous carcinoma tissue and adjacent non-neoplastic tissue specimens via immunohistochemistry. CLDNs were expressed in the cell membrane (Fig. 2). CLDN1 expression was observed in 28.8% (23/80) of laryngeal squamous carcinoma tissues and in 61.3% (49/80) of adjacent non-neoplastic tissues (P<0.01; Fig. 2A and B; Table I). As presented in Table I, the expression of CLDN1 was not associated with age (P>0.999), gender (P>0.999), the expression of Ki67 (P>0.999), histological grade (P>0.999) or distant metastasis (P=0.296).

The membrane staining of CLDN3 and CLDN8 was increased in laryngeal squamous carcinoma tissues compared with adjacent non-neoplastic tissues (Fig. 2). CLDN3 was expressed in 67.5% (54/80) of laryngeal squamous carcinoma tissues and in 30.0% (24/80) of adjacent non-neoplastic tissues (P<0.01; Fig. 2C and D; Table II). As presented in Table II, the expression of CLDN3 was not associated with gender (P=0.243), age (P=0.276), histological grade (P>0.999) or the expression of Ki67 (P=0.175), but was associated with distant metastasis (P<0.01).

Membrane expression of CLDN7 protein was observed in 22.5% (18/80) of laryngeal squamous carcinoma tissues and in 65.0% (52/80) of adjacent non-neoplastic tissues (P<0.01; Fig. 2E and F; Table I). As presented in Table I, the expression of CLDN7 was not associated with age (P=0.462), gender (P>0.999), histological grade (P>0.999) or the expression of Ki67 (P=0.496), but was associated with distant metastasis (P<0.01). The results suggested that CLDN1 and CLDN7 are downregulated in laryngeal squamous carcinoma.

As presented in Fig. 2G and H, the membrane staining of CLDN8 was increased in laryngeal squamous carcinoma tissues compared with in non-neoplastic tissues. CLDN8 was expressed in 48.8% (39/80) of laryngeal squamous carcinoma tissues and in 21.3% (17/80) of adjacent non-neoplastic tissues (P<0.01; Table II). As presented in Table II, the expression of CLDN8 was not associated with age (P=0.146), gender (P>0.999) or distant metastasis (P=0.124), but was associated with histological grade (P<0.05) and the expression of Ki67 (P<0.01). The

Table I. Expression of CLDN1 and CLDN7 clinicopathological characteristics of patients with laryngeal squamous carcinoma.

Factor	n	CLDN1 (+)	CLDN1 (-)	χ^2	P-value	n	CLDN7 (+)	CLDN7 (-)	χ^2	P-value
Laryngeal squamous carcinoma tissue	80	23	57	8.673	<0.01	80	18	62	9.493	<0.01
Adjacent non-neoplastic tissue	80	49	31			80	52	28		
Gender										
Male	46	13	33	0.178	>0.999[a]	46	8	38	0.164	>0.999[a]
Female	34	10	24			34	10	24		
Age (years)										
≤60	58	15	43	0.116	>0.999[a]	58	14	44	0.422	0.462[a]
>60	22	8	14			22	4	18		
Histological grade										
Well- differentiated	42	14	28	0.243	>0.999[a]	42	10	32	0.096	>0.999[a]
Moderately and poor differentiated	38	9	29			38	8	30		
Distant metastasis										
+	47	13	34	1.568	0.296[a]	47	6	41	9.286	<0.01
-	33	10	23			33	12	21		
Ki67										
+	27	7	20	0.234	>0.999[a]	29	7	20	0.448	0.496[a]
-	53	16	37			43	11	42		

[a]No statistical significance. CLDN, claudin.

Table II. Expression of CLDN3 and CLDN8 and clinicopathological characteristics of patients with laryngeal squamous carcinoma.

Factor	n	CLDN3 (+)	CLDN8 CLDN3 (-)	χ^2	P-value	n	(+)	CLDN8 (-)	χ^2	P-value
Laryngeal squamous carcinoma tissue	80	54	26	8.078	<0.01	80	39	41	9.451	<0.01
Adjacent non-neoplastic tissue	80	24	56			80	17	63		
Gender										
Male	46	28	18	1.216	0.243[a]	46	23	23	0.112	>0.999[a]
Female	34	26	8			34	16	18		
Age (years)										
≤60	58	40	18	1.382	0.276[a]	58	27	31	2.218	0.146[a]
>60	22	12	10			22	12	10		
Histological grade										
Well- differentiated	42	28	14	0.124	>0.999[a]	42	16	26	4.326	<0.05
Moderately and poor differentiated	38	26	12			38	23	15		
Distant metastasis										
+	47	37	10	9.624	<0.01	47	20	27	0.943	0.124[a]
-	33	17	16			33	19	14		
Ki67										
+	27	20	7	1.023	0.175[a]	27	18	9	8.652	<0.01
-	53	34	19			53	21	32		

[a]No statistical significance. CLDN, claudin.

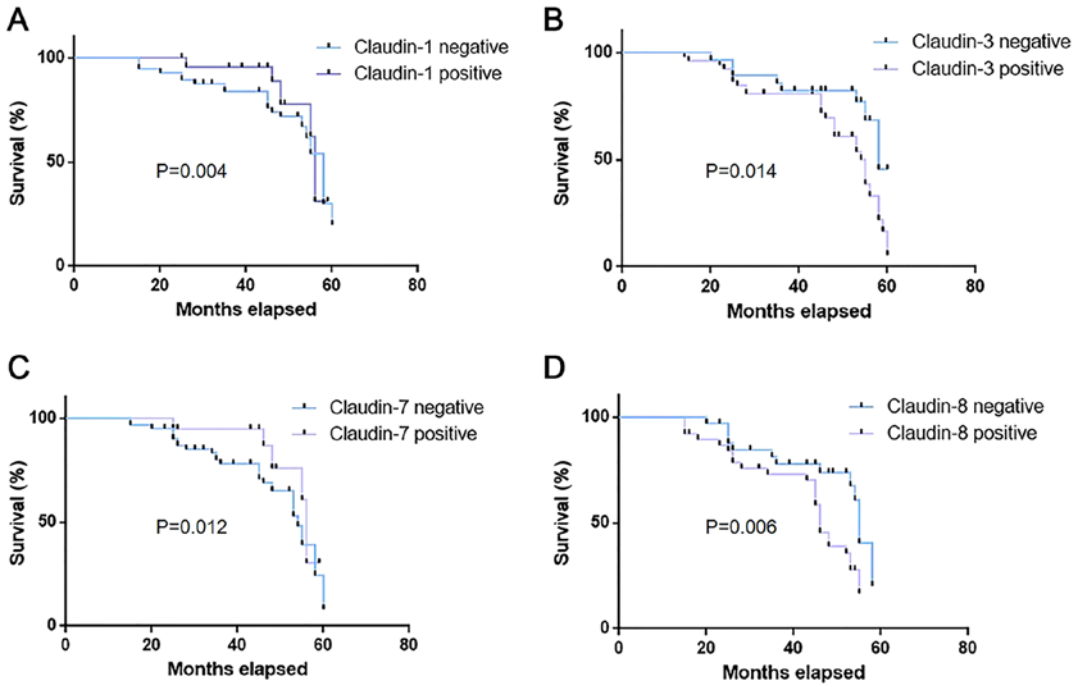

Figure 3. Association between the expression of CLDNs and patient survival. Kaplan-Meier analysis was performed to determine the survival of patients with laryngeal squamous carcinoma. Survival was investigated according to (A) CLDN1 (P=0.004), (B) CLDN3 (P=0.014), (C) CLDN7 (P=0.012) and (D) CLDN8 expression (P=0.006). CLDN, claudin.

Table III. Association between the levels of CLDN1 and CLDN7 expression in laryngeal squamous carcinoma tissues.

Expression	CLDN7 (+)	CLDN7 (-)	φ	P-value
CLDN1 (+)	14	9	0.897	<0.01
CLDN1 (-)	4	53		

CLDN, claudin.

results suggested that CLDN3 and CLDN8 are upregulated in laryngeal squamous carcinoma.

CLDN1 and CLDN7 are concurrently expressed in laryngeal squamous carcinoma tissue. As presented in Table III, a significant association between CLDN1 and CLDN7 expression was observed in laryngeal squamous carcinoma tissues (φ=0.897, P<0.01).

Associations with survival and clinical outcomes. As presented in Fig. 3, patients with tumors that were positive for CLDN1 and CLDN7 (median survival, 51.42 and 52.43 months, respectively) exhibited significantly increased survival times (P=0.004 and P=0.012, respectively) compared with those negative for CLDN1 and CLDN7 (median survival, 45.29 and 45.56 months, respectively). Patients with tumors that were positive for CLDN3 and CLDN8 (median survival, 44.32 and 44.87 months, respectively) exhibited significantly reduced survival times (P=0.014 and P=0.006, respectively) compared with those negative for CLDN3 and CLDN8 (median survival, 52.27 and 52.13 months, respectively). The results indicated that the expression profiles of CLDNs are associated with the survival of patients with laryngeal squamous carcinoma.

Discussion

Alterations in cell-to-cell adhesion are commonly reported as early events in metastasis, permitting the release of individual tumor cells from the primary tumor (6). Cell-to-cell adhesion of epithelial cells is primarily maintained via adherens junctions and TJs (1,3). Studies have previously investigated the adherens junction protein E-cadherin (38); deficiencies in E-cadherin function have been reported to lead to an enhancement in cell motility and confer invasive abilities in various cell types (39,40). Thus, E-cadherin is considered a tumor suppressor in a variety of tissues and has been demonstrated to be a valuable prognostic marker for numerous human cancers (41), indicating the important role of cell-to-cell adhesion proteins in tumorigenesis.

Interruption of TJs, which serve crucial roles in the penetrability and polarity of cells, is hypothesized to lead to epithelial tumorigenesis (42). Alterations in the structure and function of TJs have been reported in numerous types of tumor (22). Dysregulated expression of CLDN proteins may lead to the disruption of TJs, and subsequently affect the polarity and interconnectivity of cells (21). Reduced or aberrant expression of CLDNs has been hypothesized to be associated with pathophysiological consequences (43). CLDN1-deficient mice exhibit lethality 1 day following birth due to the loss of epidermal barrier function (44). Abnormalities in TJ integrity induced by dysregulated CLDN expression may serve major roles in permitting the dispersion of nutrients and other factors essential for the maintenance and progression of tumor cells (45). Disruption of CLDNs in tumors has been suggested to be a mechanism underlying reduced cell adhesion and an important event in the

progression of tumor cells toward metastasis (46-48). Consistent with this theory, a previous study demonstrated that the expression of CLDN4 in pancreatic carcinoma cells decreased the metastatic phenotype of these cells (49). Additionally, CLDN1 overexpression in tumor cells promotes the apoptosis of cells in three-dimensional cultures (50). The physiological ratio of CLDNs serves an important role in maintaining the structure and function of TJs in epithelial cells (19); however, the mechanisms by which altered CLDN expression and damage to TJs enhance tumor formation, and the effects of these alterations on the progression of tumors remain unclear.

The primary cause of cancer-associated mortality is malignancy due to the metastasis of tumor cells from primary tumor locations to distant organs (21). An important step in the metastasis of tumor cells is hypothesized to be the epithelial-mesenchymal transition (EMT) (51,52). Downregulation of certain CLDNs in tumors is associated with the interruption of TJs during tumorigenesis and EMT (53,54). Furthermore, enforced initiation of EMT in epithelial cells leads to loss of function of TJs and abnormal expression of CLDNs (55). Additionally, CLDNs have been revealed to interact with the TJ protein zonula occludens-1 (ZO-1) via their C-termini (15). Notably, ZO-1 binds to numerous proteins that are involved in cell signaling and transcriptional regulation (56,57), suggesting that CLDNs may serve roles in these processes. Providing that CLDN expression profiles are tumor-specific, it has been proposed that CLDNs may be valuable biomarkers for various types of tumor. For example, a set of four indicators that included CLDN3 was reported as an effective biomarker to precisely classify 158 cases of ovarian cancer (58). Furthermore, CLDNs may serve as prognostic markers. CLDN1 expression is associated with the poor prognosis of stage II colon cancer (59). In addition, CLDN10 expression has been reported to be an autonomous prognostic indicator of hepatocellular carcinoma recurrence (60); however, at present, the association between laryngeal squamous carcinogenesis and the expression of CLDNs remains unclear. In the present study, the expression of CLDNs was determined in laryngeal squamous carcinoma samples and adjacent non-neoplastic tissues from 80 patients. It was revealed that the levels of CLDN1 and CLDN7 expression were decreased in laryngeal squamous carcinoma compared with adjacent non-neoplastic tissues, whereas those of CLDN3 and CLDN8 were increased. CLDN3 was the most commonly expressed of these proteins in laryngeal squamous carcinoma tissue; 67.5% of laryngeal squamous carcinoma cases exhibited CLDN3 reactivity, whereas the expression of CLDN1, CLDN7 and CLDN8 was observed in 28.8, 22.5 and 48.8% of cases, respectively. The functional roles of CLDN1, -3, -7 and -8 in laryngeal squamous carcinoma are yet to be determined; however, based upon their roles in cell-to-cell adhesion, abnormalities in these proteins may contribute to metastasis.

In conclusion, the present study revealed that the expression of CLDN1, 3, -7 and -8 varied between human laryngeal squamous carcinomas and adjacent non-neoplastic tissues, and expression of these proteins was associated with the survival of patients. Additionally, CLDN3 and CLDN7 expression was associated with distant metastasis. Furthermore, CLDN1 and CLDN7 were reported to exhibit significant co-expression in laryngeal squamous carcinoma tissues; however, the mechanisms underlying these observations require further investigation.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

SZ and XP performed the experiments. CW and RW contributed to the conception and design of the study. ZS revised the manuscript critically and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The present study was approved by the Ethics Committee of Jilin Cancer Hospital (approval no. JLCH01224). Informed consent for participation was obtained from all patients and/or their parents.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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