Annals of Diagnostic Pathology 50 (2021) 151673

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Review Article

The significance of tyrosine kinase receptor B and brain-derived neurotrophic factor expression in salivary duct carcinoma

Yusuke Kondo [a,*], Kenichi Hirabayashi [a], Joaquim Carreras [a], Keiichi Tsukinoki [b], Yoshihide Ota [c], Kenji Okami [d], Naoya Nakamura [a]

[a] Department of Pathology, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan
[b] Department of Oral Science, Graduate School of Dentistry, Kanagawa Dental University, Yokosuka, Kanagawa 238-8580, Japan
[c] Department of Oral and Maxillofacial Surgery, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan
[d] Department of Otolaryngology, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan

ARTICLE INFO

Keywords:
Salivary duct carcinoma
De novo
Ex-pleomorphic adenoma
Tyrosine kinase receptor B
Brain-derived neurotrophic factor
Neurotrophin-4

ABSTRACT

Salivary duct carcinoma (SDC) is a high-grade salivary gland neoplasm. It may occur *de novo* or secondarily from pleomorphic adenoma (ex-PA), with secondary development accounting for more than 50% of the cases. In recent years, the expression of tyrosine kinase receptor B (TrkB), which is in the same family as HER2, has been confirmed in various types of carcinomas. However, there are a few studies on SDC. In order to examine the expression and role of TrkB in SDC, we investigated it. Immunohistochemistry was used to detect the expression of TrkB and its ligands, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) in 20 patients with SDC. The mRNA levels of TrkB, BDNF, and NT-4 were analyzed using quantitative polymerase chain reaction. TrkB was negative in 10 cases and positive in 10 cases, BDNF was negative in 11 cases and positive in 9 cases, and NT-4 was positive in all cases. There was a high number of TrkB-positive cases in the pT4 group and The H-score of TrkB was also significantly higher in the stage III and IV groups. There was a high number of BDNF-positive cases in the ex-PA group and Histo-score of BDNF had a trend of high expression in ex-PA. There were no significant differences or correlations in mRNA expression. Our results suggest that TrkB may be involved in SDC tumor growth.

1. Introduction

Salivary duct carcinoma (SDC) is a high-grade tumor with the poorest prognosis among malignant salivary gland neoplasms [1,2]. SDC may occur *de novo* or secondarily from pleomorphic adenoma (ex-PA), and more than 50% of SDC are ex-PA [3]. The expression of human epidermal growth factor receptor 2 (HER2) and androgen receptor (AR) is observed in 21% to 83% and 64% to 100% of SDC cases, respectively [1,4-6]. Immunohistochemical examination of HER2 and AR has improved accuracy of pathological diagnosis of SDC. HER2-targeting drugs have been tested, and although the overall response rate for these drugs is reported to be 70%, there are many adverse events [7,8]. At present, there is a need for the development of targeted drugs with few adverse events and high response rate.

The expression and function of tyrosine kinase receptor B (TrkB), a tyrosine kinase-type receptor similar to HER2, has been studied in many cancer such as breast, lung, pancreatic, and cervical cancers [9-14]. In addition, brain-derived neurotrophic factor (BDNF) and its ligand TrkB are co-expressed in many carcinomas and normal tissues. TrkB signaling is triggered by autocrine signals [11,15,16].

The tumor characteristics of SDC are similar to ductal carcinoma of the breast [2,17]. Heather C et al. reported that TrkB-T1, a splicing variant of TrkB, is predominantly expressed in ductal carcinoma of the breast [9]. Vanhecke E et al. reported that BDNF is also expressed by autocrine secretion, and they suggested that TrkB signaling suppresses apoptosis of breast cancer cells and promotes survival of cancer cells by BDNF binding to TrkB-T1 [10].

Jia S et al. revealed that both TrkB and BDNF are co-expressed in adenoid cystic carcinoma (ACC) [18]. They also revealed that stage III and IV groups had significantly more BDNF- and NT-4-positive cases than stage I and II groups [18].

In SDC, Ryu HJ et al. found no cases with the expression of TrkB [19]. However, it has not been proven that TrkB is not expressed in SDC. We hypothesized that TrkB is expressed in SDC, based on the TrkB

* Corresponding author at: Department of Pathology, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan.
E-mail address: kondou@tokai-u.jp (Y. Kondo).

https://doi.org/10.1016/j.anndiagpath.2020.151673

Available online 23 November 2020

expression observed in ductal carcinoma of breast cancer and the results of our preliminary experiments [9,10,18]. Therefore, in this study, we analyzed the expression and of TrkB and its ligands, BDNF and Neurotrophin-4 (NT-4) at protein and mRNA levels in 20 SDC cases. As a result, expression of these molecules was confirmed in SDC for the first time, suggesting that TrkB may be involved in tumor growth.

2. Materials and methods

2.1. Specimens and materials

We examined 20 specimens of SDC in the Tokai University Hospital from January 2001 to August 2016. All specimens were diagnosed according to the SDC definition of the World Health Organization. SDC with residual PA was named as ex-PA. The specimens were separated into several groups described below. The clinical data were obtained from electronic medical records. Paraffin-embedded blocks were prepared by fixing the excised specimens in 10% formalin for 24 h. The blocks were cut into 4 μm thick slices, which were then stained with hematoxylin and eosin, and prepared for immunohistochemistry and quantitative polymerase chain reaction (qPCR).

2.2. Immunohistochemistry

The sections were de-paraffinized with xylene and rehydrated in ethanol series (100 to 70%). The tissue sections were exposed to anti-TrkB (sc-8316, 1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-BDNF (ab108319, 1:500, Abcam, Cambridge, UK), and anti-NT-4 (NBP1-47897, 1:75, Novus Biologicals, CO, USA), Androgen receptor (AR, NCL-L-AR-318, 1:40, Leica Biosystems, Germany), HER2 (790-2991, predilution, Roche Diagnostics K.K., Switzerland), Epidermal growth factor receptor (EGFR, 423701, 1:2, NICHIREI BIOSCIENCES INC., Japan), Phosphatase and tensin homolog (PTEN, M3627, 1:50, Dako, Denmark), Pleomorphic adenoma gene 1 (PLAG1, H00005324-M02, 1:400, Abnova CO, Taiwan) and High-mobility group AT-hook 2 (HMGA2, NBP2-43640, 1:150, Novus Biologicals, CO, USA) antibodies. The staining protocol was set as the standard automation program on BOND-MAX (Leica Biosystems, Tokyo, Japan).

The intensity of fluorescence of TrkB, BDNF, NT-4 in carcinoma cells was scored as 0, 1+, 2+ or 3+ for each protein: 0, extremely feeble or no staining; 1+, mildly stained; 2+, moderately stained; 3+, strongly stained (Supplementary Fig. S1). The area occupied by each score was measured every 20 SDC cases. The coverage of 3+ and 2+ was regarded as positive. For three proteins, the number of positive cases and the positive cutoff rate were evaluated at 10% positive rate increments (10%, 20%,...,100%). In addition, the Histo-score (H-score) of TrkB, BDNF, and NT-4 was calculated using the following formula: H-Score = 1 × (% cells 1+) + 2 × (& cells 2+) + 3 × (% cells 3+) [20,21]. HER2 was rated 0–3 + according to ASCO/CAP guidelines.

2.3. qPCR

Total RNA was extracted from a paraffin block using an AllPrep DNA/RNA FFPE Kit (Qiagen, Valencia, CA, USA). cDNA synthesis from total RNA was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) as per manufacturer's instructions. The reaction (StepOne, Applied Biosystems) was performed using the SYBR Green FAST assay. Reagents were adjusted following the manufacturer's instructions. Cycle conditions were as follows: 20 s at 95 °C, 3 s at 95 °C and 30 s at 60 °C for 40 cycles, 15 s at 95 °C, 1 s at 60 °C and 15 s at 95 °C (melt curve).

The target genes primer sequences were: TrkB full length (FL), forward 5′-GGAGTAACACTCCATCTTCTTCG-3′ and reverse 5′-TGGTGATGCCAAAGTACTGG-3′; TrkB truncated 1(T1), forward 5′-TAAAACCGGTTGGGAACATC-3′ and reverse 5′-ACCCATCCAGTGGGATCTTA-3′; BDNF, forward 5′-AACCTTCTGCCCATCCTGT-3′ and reverse 5′-GCTCCCAACTTGACTTCTCC-3′; NT-4, forward 5′-AGGCCAAGCAGTCCTATGT-3′ and reverse 5′-GGTCTCTCAGCATCCAGCT-3′, glyceraldehyde 3-phosphate dehydrogenase, forward 5′-CAAATTCCATGGCACCGTC-3′ and reverse 5′-TCTCGCTCCTGGAAGATGGT-3′. Gene expression values were normalized to endogenous control GAPDH. Each experiment was performed in triplicate. Mean triplicate Ct values for each sample were calculated, and outliers within the triplicates removed. The ΔCt was calculated by subtracting the mean GAPDH Ct from the mean target Ct, and then the relative expression is calculated using the formula $2^{-\Delta Ct}$.

2.4. Statistical analyses

The results were analyzed using SPSS version 24.0 (IBM, Chicago, IL, USA). Depending on the type of data, the chi-square test, Mann-Whitney *U* test, Wilcoxon test, Fisher's exact test and TwoStep cluster analysis were performed. A *p*-value of 0.05 or less was considered significant.

3. Results

3.1. Clinicopathological findings

The clinicopathological findings of the patients are shown in Table 1. The patients consisted of 19 males and one female, and the median age was 51 years, with a range of 34 to 84. There were 15 parotid gland and 5 submandibular gland cases. The median tumor size was 3.25 cm (ranging from 1.5 to 6.5 cm). There were 8 cases of *de novo* and 12 cases of ex-PA. pT1 was found in 2 cases, pT2 in 3 cases, pT3 in 7 cases, and pT4 in 8 cases. Lymph node metastasis (N) was observed in 16 cases. Distant metastasis (M) was observed in 1 case. Stage II was found in 2 cases, Stage III in 1 case, Stage IVA in 16 cases and Stage IVC in 1 case (Table 1).

3.2. Protein expression of TrkB, BDNF, NT-4, AR, HER2, EGFR, PTEN PLAG1 and HMGA2

The cutoff value was set to 10% for TrkB, and 30% for both BDNF and NT-4, and regarded as low expression and overexpression, respectively.

TrkB was negative in 10 cases and positive 10 cases. The mean positive percentage of stained cells was 1.0% in the negative cases and 32.0% in the positive cases (Tables 2 and 3). The H-Score of TrkB had a median of 85 with a range of 0 - 180 (Table 2). TrkB was primarily positive in the cytoplasm (Fig. 1A-1 and A-2). Intra-tumor heterogeneity was noted (Fig. 2). The highest score of the positive cases was 2+, with no cancer cells showing 3+.

BDNF was negative in 11 cases and positive in 9 cases. The mean percentage of stained cells was 9.5% for the negative cases and 63.9% for the positive cases (Tables 2 and 3). The H-Score of BDNF had a median of 120 with a range of 80 - 180 (Table 2). BDNF was positive in the cytoplasm (Fig. 1B-1 and B-2). In ex-PA, the PA component showed scores of 2+ to 1+ regardless of the results of BDNF at the SDC component (Fig. 3).

NT-4 was positive in all cases. The mean percentage of stained cells was 61.8% for the positive cases (Tables 2 and 3). The H-Score of NT-4 had a median of 170 with a range of 95 - 190 (Table 2). NT-4 was positive in the cytoplasm (Fig. 1C-1 and C-2). The number of NT-4 positive cases was higher than the BDNF-positive ones.

AR was positive in 18 cases of 20 cases (90%). HER2 showed score 0 in 2 cases, 1+ in 8 cases, 2+ in 1 case, and 3+ in 9 cases. EGFR was positive in 9 cases (45%) and PTEN was positive in 10 cases (50%). PLAG1 was positive in 4 cases of 20 cases (20%), all of which were ex-PA. HMGA2 was positive in 14 cases of 20 cases (70%: ex-PA, 11/12 and *de novo*, 3/8 cases.) (Table 2).

Table 1
Clinical features of 20 patients with SDC.

Case no.	Age	Sex	Region	Size (cm)	De novo/ Ex-PA	pT	N	M	Stagae
1	48	m	PG	3	Ex PA	pT2	N0	M0	II
2	65	m	PG	2	Ex PA	pT4a	N2b	M0	IVA
3	67	m	PG	2	De novo	pT1	N2b	M0	IVA
4	34	m	PG	6.5	Ex PA	pT3	N2b	M0	IVA
5	39	m	PG	5.5	De novo	pT4a	N2b	M0	IVA
6	56	m	PG	2.2	Ex PA	pT2	N0	M0	II
7	61	m	PG	3	Ex PA	pT3	N2b	M0	IVA
8	60	m	PG	2.5	Ex PA	pT4a	N2b	M0	IVA
9	61	m	SMG	4.2	Ex PA	pT4a	N2c	M1	IVC
10	81	m	SMG	6	Ex PA	pT3	N2b	M0	IVA
11	76	m	PG	5	De novo	pT3	N2b	M0	IVA
12	63	m	SMG	1.5	de novo	pT1	N2b	M0	IVA
13	78	m	PG	3.5	Ex PA	pT2	N2b	M0	IVA
14	58	m	PG	2.5	De novo	pT4a	N0	M0	IVA
15	52	f	PG	5	Ex PA	pT3	N2b	M0	IVA
16	63	m	PG	5	Ex PA	pT4a	N2b	M0	IVA
17	53	m	PG	2.8	De novo	pT3	N1	M0	III
18	84	m	SMG	5.5	De novo	pT3	N2b	M0	IVA
19	58	m	SMG	6	Ex PA	pT4a	N0	M0	IVA
20	81	m	PG	3	De novo	pT4a	N1	M0	IVA

m, male; f, female; PA, pleomorphic adenoma; PG, palotid gland; SMG, submandibular gland.

Table 2
Results of immunohistochemistry.

Case no.	Coverage of 2+ and 3+						H-score			AR	HER2	PLAG1	HGMA2	EGFR	PTEN
	TrkB	Cutoff 10%	BDNF	Cutoff 30%	NT-4	Cutoff 30%	TrkB	BDNF	NT-4						
1	0	−	45	+	60	+	0	110	160	+	3+	+	+	+	+
2	40	+	50	+	70	+	140	150	180	+	2+	−	+	−	+
3	0	−	60	+	40	+	100	160	135	+	3+	−	+	+	+
4	0	−	60	+	40	+	5	160	140	+	3+	+	+	−	−
5	10	+	10	−	25	+	60	110	95	+	3+	−	−	−	−
6	0	−	60	+	50	+	10	160	150	+	0	−	+	−	+
7	40	+	70	+	50	+	140	170	155	+	1+	−	+	−	−
8	0	−	5	−	70	+	30	105	170	+	3+	+	+	−	−
9	20	+	80	+	60	+	120	180	160	+	1+	−	−	+	−
10	5	−	70	+	70	+	95	170	170	+	3+	−	+	+	−
11	0	−	20	−	80	+	95	120	180	+	3+	−	+	+	+
12	0	−	10	−	90	+	95	80	170	+	1+	−	−	+	+
13	10	+	20	−	70	+	80	120	170	+	1+	−	+	−	−
14	80	+	20	−	70	+	180	120	170	+	1+	−	−	−	+
15	0	−	80	+	65	+	0	180	170	+	3+	−	+	−	+
16	10	+	0	−	80	+	50	100	190	+	1+	−	+	−	−
17	80	+	0	−	70	−	170	100	170	+	1+	−	−	+	−
18	5	−	20	−	55	+	50	120	145	−	1+	−	+	−	+
19	20	+	0	−	50	+	90	100	140	+	3+	+	+	+	+
20	10	+	0	−	70	−	70	100	170	−	0	−	−	+	−

H-score, Hist-score.

Table 3
Summary of immunohistochemistry of TrkB, BDNF and NT-4.

	Number of negative case	Number of positive case
	Mean of positive rate	Mean of positive rate
TrkB	10	10
	1.0%	32.0%
BDNF	11	9
	9.5%	63.9%
NT-4	0	20
	–	61.8%

3.3. Clinicopathological relevance

Expression of TrkB in the pT4 group (1 negative case and 7 positive cases) was significantly higher than that of the pT1-3 group (9 negative and 3 positive cases) ($p = 0.010$, Fisher's exact test, Fig. 4). The H-score of TrkB in cases of Stages III and IV was significantly higher than that in cases of Stages I and II ($p = 0.050$, Kruskal-wallis test, Supplementary Fig. S2). TrkB, pT, pN, and M were analyzed using the TwoStep cluster analysis; results were classified into two clusters based on the results for TrkB (Table 4). The quality of the analysis was "good"; therefore, the results were considered reliable (Supplementary Fig. S3). Expression of BDNF in ex-PA cases (4 negative and 8 positive cases) was significantly higher than that of the *de novo* cases (7 negative cases and 1 positive case) ($p = 0.025$, Fisher's exact test) (Fig. 5). H-score of BDNF had a trend of high expression in ex-PA to compared with *de novo* ($p = 0.086$, Kruskal-wallis test, Supplementary Fig. S2). BDNF-positive cases tended to be HMGA2-positive ($p = 0.083$, Likelihood Ratio). Both PLAG1 and HMGA2 had significantly more positive cases of ex-PA than *de novo* (PLAG1, $p = 0.029$; HMGA2, $p = 0.008$, Likelihood ration). There were no significant findings related to NT-4.

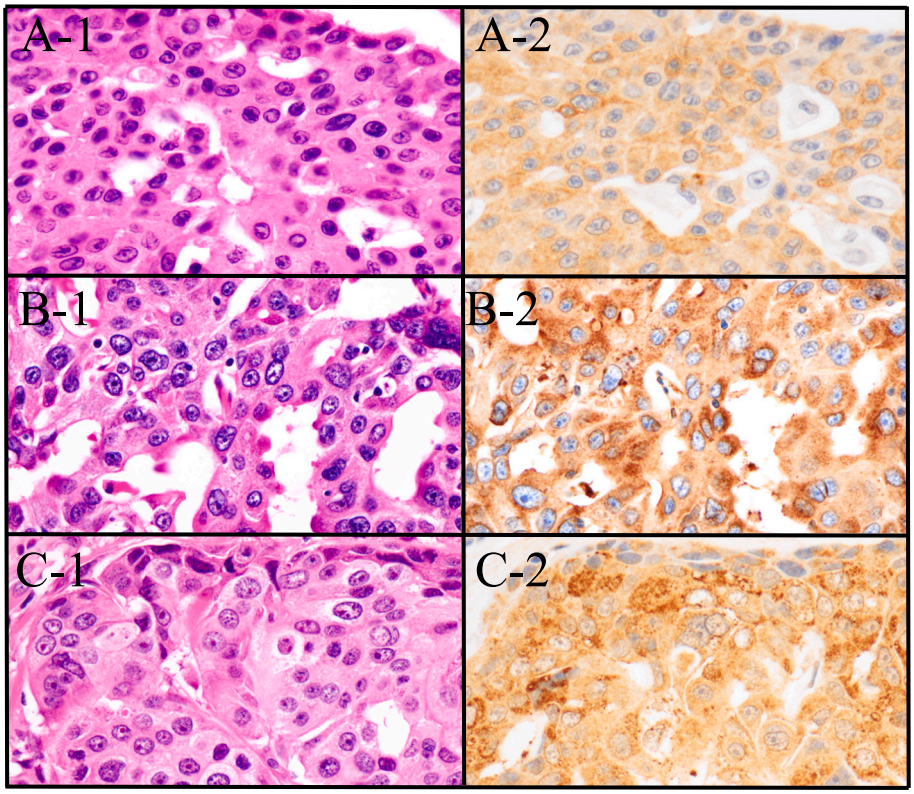

Fig. 1. Immunohistochemistry of TrkB, BDNF and NT-4.
Cytoplasm of all cells were positive for (A-1,2) TrkB, (B-1,2) BDNF, and (C-1,2) NT-4.

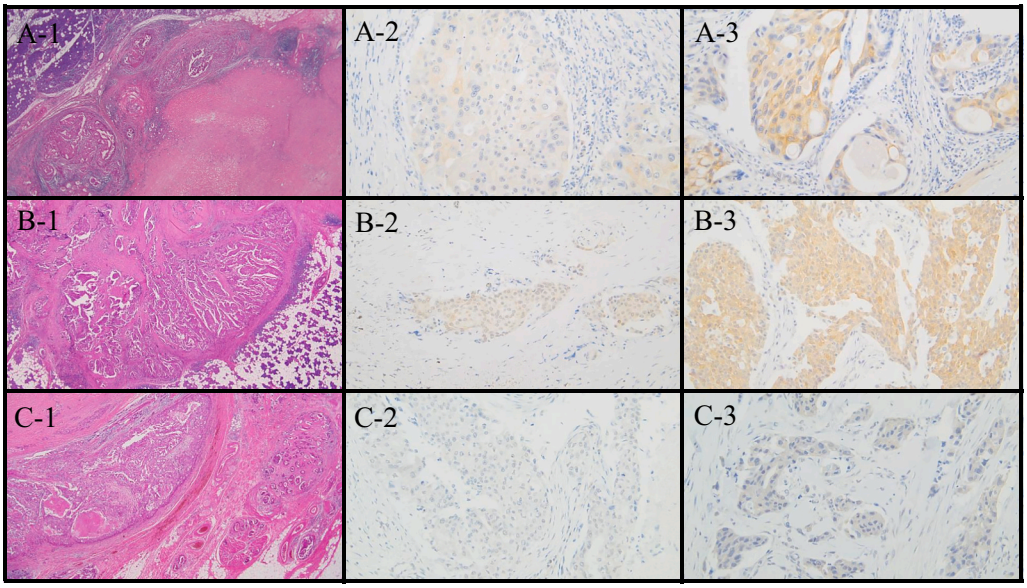

Fig. 2. Immunohistochemistry of TrkB. The highest score was 2+ in TrkB-positive cases and no cancer cells were scored 3+. (A-1) Case 7, TrkB+. (A-2) 1+, 60%. (A-3) 2+, 40%. (B-1) Case 14, TrkB+. (B-2) 1+, 20%. (B-3) 2+, 80%. (C-1) Case 8, TrkB-. (C-2) 0, 70%. (C-3) 1+, 30%.
*0, extremely feeble or no staining; 1+, mild staining; 2+, moderate staining; 3+, strong staining.

3.4. TrkB, BDNF, and NT-4 mRNA expression levels

There was no significant difference in the mRNA expression levels between TrkB-FL and TrkB-T1. There was also no significant difference between the pT1–3 and the pT4 groups in terms of TrkB-FL and TrkB-T1 (Supplementary Fig. S4). There was no significant difference in BDNF mRNA expression levels between *de novo* and ex-PA cases (Supplementary Fig. S5). No correlation was noted in the immunohistochemistry results for any of the mRNAs expression levels (data not shown).

4. Discussion

In this study, we demonstrated that TrkB was expressed in SDC. To the best of our knowledge, this is the first report to show the expression of TrkB in SDC. There is one previous study that showed negative TrkB expression in SDC, which differs from our results [19]. We applied the use of antibody with different epitopes to our study. As TrkB has a few variants, some antibodies may give negative results depending on the epitope. We used antibodies that bind to the extracellular domain

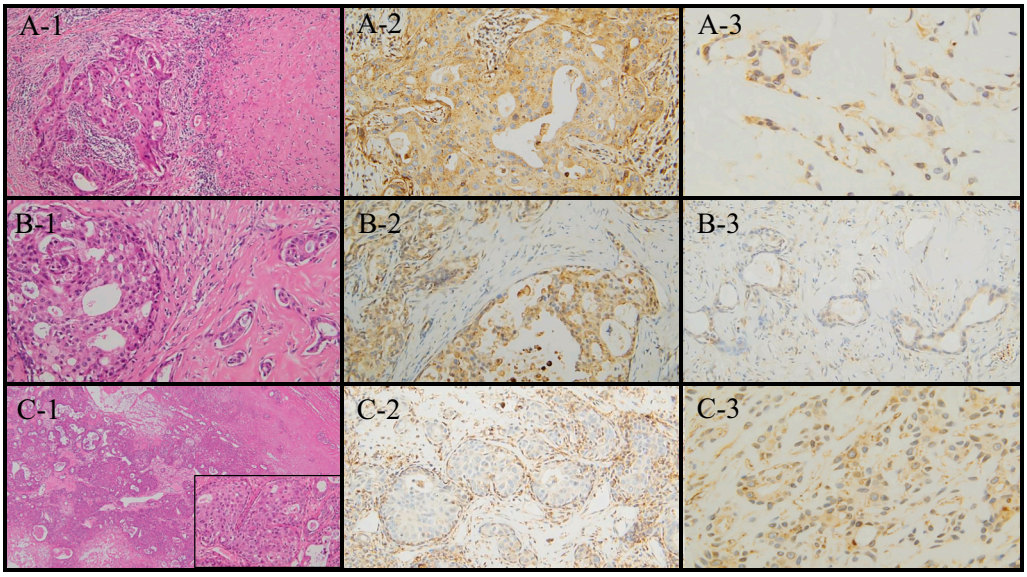

Fig. 3. Immunohistochemistry of BDNF.
In ex-PA case, PA showed a score of 1+ or 2+ regardless of the expression level of BDNF in SDC. (A-1) Case 7, BDNF+. (A-2) SDC com., 2+ 70%. (A-3) PA com., 2 + .
(B-1) Case 6, BDNF+. (B-2) SDC com., 2+ 60%. (B-3) PA com., 1+ - 2+.
(C-1) Case 19, BDNF-, inset, SDC com. (C-2) SDC com., 1+/0, 100%. (C-3) PA com., 2 + .
*com., component; PA, Pleomorphic adenoma.
**,*0, extremely feeble or no staining; 1+, mild staining; 2+, moderate staining; 3+, strong staining.

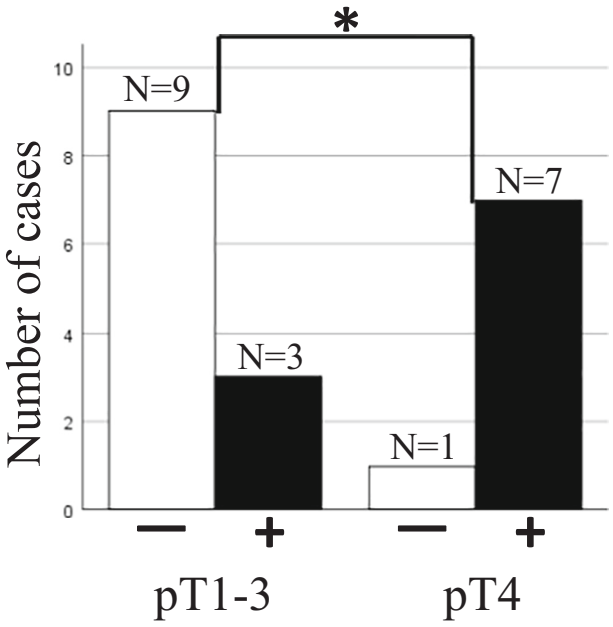

Fig. 4. Statistical analysis results of TrkB and pT.
Between the pT1-3 group and the pT4 group, the latter had significantly more TrkB-positive cases.
*$p = 0.010$ (Fisher's exact test).

Table 4
Results of TwoStep cluster analysis.

Importance	Number of case		Cluster 1	Cluster 2
			10	10
High	TrkB	+	0	10
		−	10	0
	pT	pT4	1	7
		pT3	5	2
		pT2	2	1
		pT1	2	0
	pN	N2c	0	1
		N2b	8	5
		N1	0	2
		N0	2	2
Low	M	M1	0	1
		M0	10	9

(amino acids 160–340) that exist in all TrkB variants. Thus, we demonstrated TrkB expression in SDC, which is a new finding. TrkB expression was significant in the pT4 group compared to the pT1–3 group. In the TwoStep cluster analysis, results were classified into two clusters based on TrkB. SDC also has an anti-apoptotic effect due to TrkB signaling similar to breast cancer [10]. Therefore, we believe that TrkB may be greatly involved in SDC development.

We confirmed BDNF expression in SDC. This result suggests that BDNF and TrkB are co-expressed in SDC as in other tumors, and that TrkB signaling is triggered by autocrine signaling [10,15,16]. In our study, BDNF expression was significantly higher in ex-PA cases than *de novo* cases, and H-score of BDNF had a trend of high expression in ex-PA. BDNF was expressed in the PA component regardless of the BDNF expression in the SDC component. Additional immunohistochemistry showed that BDNF was also positive for carcinoma-ex-PA, *in situ* carcinoma-ex-PA, Cellar PA and Atypical PA (Supplementary Fig. S6). These are new findings. We consider that BDNF expression is one of the phenotypes of PA and that it is inherited in ex-PA [22-24]. Furthermore, we consider that BDNF expression in immunohistochemistry can be used to differentiate ex-PA from *de novo*.

Our study confirmed the expression of NT-4, but this is not surprising, as NT-4 is also secreted as a response to autocrine signaling, as well as BDNF and TrkB [25,26]. NT-4 may also bind to TrkB and induce a signaling pathway in the same manner as BDNF. In this study, the number of NT-4 positive cases was higher than that of BDNF, which suggests that NT-4 is more constantly secreted than BDNF, regardless of tumor size, *de novo* or ex-PA cases. NT-4 may be affecting SDC more than BDNF.

Amplification of TrkB-FL, TrkB-T1, BDNF and NT-4 mRNA expression was confirmed in SDC, but no correlation between immunohistochemistry results and clinicopathological findings was found. We believe that the expression of TrkB, BDNF and NT-4 may be affected by the proteasome [27-30]. We will further investigate this with cell culture experiments.

In conclusion, the expression of TrkB, BDNF and NT-4 in SDC was confirmed for the first time. In particular, TrkB expression may be involved in tumor growth in SDC.

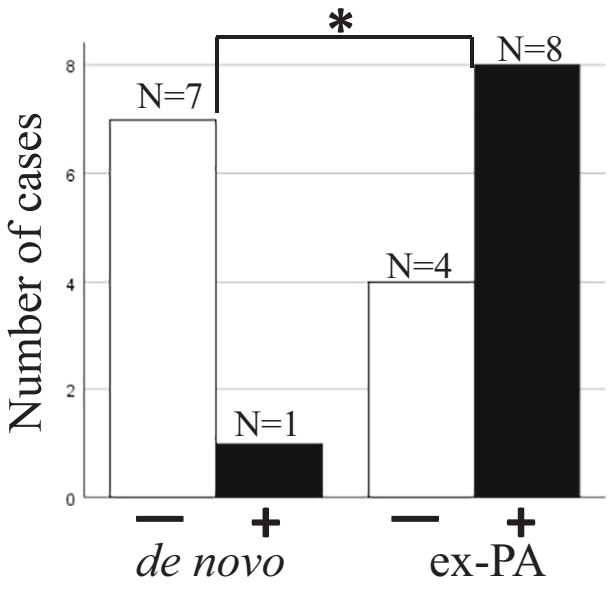

Fig. 5. Results of BDNF and *de novo*/ex-PA statistical analysis.
Between the *de novo* group and the ex-PA group, the latter had significantly more BDNF positive cases. $*p = 0.025$ (Fisher's exact test) PA, Pleomorphic adenoma.

Ethics

The present study was approved by the clinical research review committee established by the Tokai University Hospital (16R-125).

Funding

This research was funded by KAKENHI Grant-in-Aid for Young science [B, grant number 16K19089] from the Japan Society for the Promotion of Science.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We would like to thank Moemi Yoshimura, Yumi Takanashi, Yara Yukie Kikuti of the Department of Pathology at Tokai University School of Medicine; Keiko Yokoyama, Masayuki Tanaka of Support Center for Medical Research and Education at Tokai University; Tsubasa Saika Akihiko Serizawa Diagnostic Pathology Center, Tokai University Hospital; and Hirotaka Kono of the Department of Pathology at Ebina General Hospital for their support with the experiments. Also we would like to thank Editage (www.editage.com) for the English language editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.anndiagpath.2020.151673.

References

[1] Santana T, Pavel A, Martinek P, Steiner P, Grossmann P, Baněčková M, et al. Biomarker immunoprofile and molecular characteristics in salivary duct carcinoma: clinicopathological and prognostic implications. Hum Pathol 2019;93:37–47. https://doi.org/10.1016/j.humpath.2019.08.009. Aug 19.

[2] Kondo Y, Kikuchi T, Esteban JC, Kumaki N, Ogura G, Inomoto C, et al. Intratumoral heterogeneity of HER2 protein and amplification of HER2 gene in salivary duct carcinoma. Pathol Int 2014;64(9):453–9. https://doi.org/10.1111/pin.12195. Sep.

[3] D'heygere E, Meulemans J, Vander Poorten V. Salivary duct carcinoma. Curr Opin Otolaryngol Head Neck Surg. 2018 Apr;26(2):142–151. doi:https://doi.org/10.1097/moo.0000000000000436.

[4] Yeoh CC, Dabab N, Rigby E, Chhikara R, Akaev I, Gomez RS, et al. Androgen receptor in salivary gland carcinoma: a review of an old marker as a possible new target. J Oral Pathol Med 2018;47(7):691–5. https://doi.org/10.1111/jop.12741. Aug.

[5] Jaehne M, Roeser K, Jaekel T, Schepers JD, Albert N, Löning T. Clinical and immunohistologic typing of salivary duct carcinoma: a report of 50 cases. Cancer 2005;103(12):2526–33. https://doi.org/10.1002/cncr.21116. Jun 15.

[6] Glisson B, Colevas AD, Haddad R, Krane J, El-Naggar A, Kies M, Costello R, Summey C, Arquette M, Langer C, Amrein PC, Posner M. HER2 expression in salivary gland carcinomas: dependence on histological subtype. Clin Cancer Res. 2004 Feb 1;10(3):944–6. doi:https://doi.org/10.1158/1078-0432.ccr-03-0253.

[7] Otsuka K, Imanishi Y, Tada Y, Kawakita D, Kano S, Tsukahara K, et al. Clinical outcomes and prognostic factors for salivary duct carcinoma: a multi-institutional analysis of 141 patients. Ann Surg Oncol 2016;23(6):2038–45. https://doi.org/10.1245/s10434-015-5082-2. Jun.

[8] Park JC, Ma TM, Rooper L, Hembrough T, Foss RD, Schmitt NC, et al. Exceptional responses to pertuzumab, trastuzumab, and docetaxel in human epidermal growth factor receptor-2 high expressing salivary duct carcinomas. Head Neck 2018;40(12):E100–6. https://doi.org/10.1002/hed.25392. Dec.

[9] Cameron HL, Foster WG. Dieldrin promotes resistance to anoikis in breast cancer cells in vitro. Reprod Toxicol 2008;25(2):256–62. https://doi.org/10.1016/j.reprotox.2007.11.013. Feb.

[10] Vanhecke E, Adriaenssens E, Verbeke S, Meignan S, Germain E, Berteaux N, Nurcombe V, Le Bourhis X, Hondermarck H. Brain-derived neurotrophic factor and neurotrophin-4/5 are expressed in breast cancer and can be targeted to inhibit tumor cell survival. Clin Cancer Res. 2011 Apr 1;17(7):1741–52. doi:https://doi.org/10.1158/1078-0432.ccr-10-1890.

[11] Tsai YF, Tseng LM, Hsu CY, Yang MH, Chiu JH, Shyr YM. Brain-derived neurotrophic factor (BDNF) -TrkB signaling modulates cancer-endothelial cells interaction and affects the outcomes of triple negative breast cancer. PLoS One 2017;12(6). https://doi.org/10.1371/journal.pone.0178173. e0178173. Jun 12.

[12] Ozono K, Ohishi Y, Onishi H, Nakamura K, Motoshita J, Kato M, et al. Brain-derived neurotrophic factor/tropomyosin-related kinase B signaling pathway contributes to the aggressive behavior of lung squamous cell carcinoma. Lab Invest 2017;97(11):1332–42. https://doi.org/10.1038/labinvest.2017.45. Nov.

[13] Sclabas GM, Fujioka S, Schmidt C, Li Z, Frederick WA, Yang W, et al. Overexpression of tropomysin-related kinase B in metastatic human pancreatic cancer cells. Clin Cancer Res 2005;11(2 Pt 1):440–9. Jan 15. [No DOI].

[14] Moon A, Won KY, Lee JY, Kang I, Lee SK, Lee J. Expression of BDNF, TrkB, and p53 in early-stage squamous cell carcinoma of the uterine cervix. Pathology 2011;43(5):453–8. https://doi.org/10.1097/pat.0b013e3283484a3a. Aug.

[15] Chen B, Liang Y, He Z, An Y, Zhao W, Wu J. Autocrine activity of BDNF induced by the STAT3 signaling pathway causes prolonged TrkB activation and promotes human non-small-cell lung cancer proliferation. Sci Rep 2016;6. https://doi.org/10.1038/srep30404. 30404. Jul 26.

[16] Kondo Y, To M, Saruta J, Hayashi T, Sugiyama H, Tsukinoki K. Role of TrkB expression in rat adrenal gland during acute immobilization stress. J Neurochem 2013;124(2):224–32. https://doi.org/10.1111/jnc.12030. Jan.

[17] Jalaly JB, Sanati S, Chernock RD, Dibe DG, El-Mofty SK. Salivary duct carcinoma and invasive ductal carcinoma of the breast: a comparative immunohistochemical study. Head Neck Pathol 2018;12(4):488–92. https://doi.org/10.1007/s12105-017-0882-2. Dec.

[18] Jia S, Wang W, Hu Z, Shan C, Wang L, Wu B, et al. BDNF mediated TrkB activation contributes to the EMT progression and the poor prognosis in human salivary adenoid cystic carcinoma. Oral Oncol 2015;51(1):64–70. https://doi.org/10.1016/j.oraloncology.2014.10.008. Jan.

[19] Ryu HJ, Koh YW, Yoon SO. The implications of TrkA and MET aberrations in *de novo* salivary duct carcinoma. Hum Pathol 2018;81:18–25. https://doi.org/10.1016/j.humpath.2018.04.027. Nov.

[20] Hirsch Fred R, Varella-Garcia Marileila, Bunn Jr Paul A, Maria Michael V Di, Veve Robert, Bremmes Roy M, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003;21(20):3798–807. https://doi.org/10.1200/jco.2003.11.069. Oct 15.

[21] John T, Liu G, Tsao M-S. Overview of molecular testing in non-small-cell lung cancer: mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors. Oncogene 2009;28(Suppl. 1):S14–23. https://doi.org/10.1038/onc.2009.197. Aug.

[22] Andreasen S, Therkildsen MH, Bjørndal K, Homøe P. Pleomorphic adenoma of the parotid gland 1985–2010: a Danish nationwide study of incidence, recurrence rate, and malignant transformation. Head Neck 2016;38(Suppl. 1):E1364–9. https://doi.org/10.1002/hed.24228. Apr.

[23] Liang Li, Williams Michelle D, Bell Diana. Expression of PTEN, androgen receptor, HER2/neu, cytokeratin 5/6, estrogen receptor-beta, HMGA2, and PLAG1 in salivary duct carcinoma. Head Neck Pathal 2019;13(4):529–34. https://doi.org/10.1007/s12105-018-0984-5. Dec.

[24] Mito Jeffrey K, Jo Vickie Y, Chiosea Simion I, Dal Cin Paola, Krane Jeffrey F. HMGA2 is a specific immunohistochemical marker for pleomorphic adenoma and

carcinoma ex-pleomorphic adenoma. Histopathology 2017;71(4):511–21. https://doi.org/10.1111/his.13246. Oct.
[25] Cortez V, Santana M, Marques AP, Mota A, Rosmaninho-Salgado J, Cavadas C. Regulation of catecholamine release in human adrenal chromaffin cells by β-adrenoceptors. Neurochem Int 2012;60(4):387–93. https://doi.org/10.1016/j.neuint.2011.12.018. Mar.
[26] Wirth MJ, Brun A, Grabert J, Patz S, Wahle P. Accelerated dendritic development of rat cortical pyramidal cells and interneurons after biolistic transfection with BDNF and NT4/5. Development 2003;130(23):5827–38. https://doi.org/10.1242/dev.00826. Dec.
[27] Pandya C, Kutiyanawalla A, Turecki G, Pillai A. Glucocorticoid regulates TrkB protein levels via c-Cbl dependent ubiquitination: a decrease in c-Cbl mRNA in the prefrontal cortex of suicide subjects. Psychoneuroendocrinology 2014;45:108–18. https://doi.org/10.1016/j.psyneuen.2014.03.020. Jul.
[28] Seo H, Kim W, Isacson O. Compensatory changes in the ubiquitin-proteasome system, brain-derived neurotrophic factor and mitochondrial complex II/III in YAC72 and R6/2 transgenic mice partially model Huntington's disease patients. Hum Mol Genet 2008;17(20):3144–53. https://doi.org/10.1093/hmg/ddn211. Oct 15.
[29] Park H, Poo MM. Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci 2013;14(1):7–23. https://doi.org/10.1038/nrn3379. Jan.
[30] Bartkowska K, Turlejski K, Djavadian RL. Neurotrophins and their receptors in early development of the mammalian nervous system. Acta Neurobiol Exp (Wars) 2010;70(4):454–67 [No DOI].