




Clinicopathological study of intraductal carcinoma of the salivary gland, with emphasis on the apocrine type

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Abstract

Intraductal carcinoma (IC) is a rare salivary gland tumor with low- to intermediate-grade cytological features. It is further classified into intercalated duct type and apocrine type based on its distinct histologic and immunohistochemical expression. Conventional salivary duct carcinoma (SDC) is an aggressive carcinoma with high-grade features and is usually associated with poor prognosis. In this study, immunohistochemistry and mutation analyses (including *HRAS/PIK3CA* mutations, *RET* rearrangement, and human epidermal growth factor receptor 2 [*HER2*] amplification) of 9 ICs (including 3 pure ICs, 6 ICs with invasive carcinoma) and 24 conventional SDCs were performed and the results were compared. Four intercalated duct-type cases were positive for SOX10 and S100 and negative for AR; five apocrine-type cases showed opposite results. All five apocrine-type cases had cysts with relatively circumscribed tumor borders and morphologically mimicking breast low-grade ductal carcinoma in situ or papillary carcinoma. *RET* fusion is detected in half of the 4 intercalated duct-type IC but not in the apocrine-type or conventional SDC. *HER2* amplification was only observed in conventional SDC. The monoclonal antibody (clone RBT-NRAS) against NRAS Q61R is a sensitive and specific marker used for detecting HRAS Q61R mutation in the salivary gland tumors. The apocrine-type IC had different cytological grades, distinct tumor growth patterns, and no evidence of low- to high-grade transition, suggesting that apocrine-type IC should be distinguished from apocrine SDC with an in situ component.

Keywords Intraductal carcinoma of the salivary gland · HRAS · PIK3CA · HER2 · Salivary duct carcinoma

Introduction

Intraductal carcinoma (IC) is the term used in the 2017 World Health Organization (WHO) Classification of Head and Neck Tumours to describe a low-grade salivary gland tumor originally called as “low-grade salivary duct carcinoma” and later re-named by WHO 2005 as “low-grade cribriform cystadenocarcinoma” [1–3]. It is further categorized as low

grade, intermediate grade, or high grade based on the degree of cytological atypia [1]. ICs are typically unencapsulated and composed of multiple cysts with intraductal proliferation of epithelial cells forming mixed cribriform, papillary, or micropapillary structures [1, 2, 4–7]. These cells are typically low-grade, cuboidal, bland ductal cells with fine chromatin, inconspicuous nucleoli, and rare mitoses [1–3]. Sometimes, an invasive component with the loss of p63-positive cells can be observed [7]. Immunohistochemically, the classical “low-grade” IC is positive for S100, SOX10, mammaglobin, and negative for androgen receptor (AR) or human epidermal growth factor receptor 2 (HER2) [4–8]. Since the immunohistochemical expression of IC is different from that of conventional salivary duct carcinoma (SDC), ICs are considered to be different types of salivary gland tumors. However, some ICs show apocrine features, increasing cytological atypia, some with nucleoli and necrosis, and strong expression of AR [4–7, 9]. Weinreb et al. first described this rare variant of IC in detail and designated it as low-grade intraductal carcinoma with marked apocrine differentiation [9]. Due to differences in cytological atypia and immunophenotypes, intraductal

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carcinoma is nowadays further described as intercalated duct type, apocrine type, or even hybrid type [5–7].

Many pathognomonic genetic changes or rearrangements have been identified in different low-grade salivary gland tumors including pleomorphic adenoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, clear cell carcinoma, secretory carcinoma, polymorphous adenocarcinoma, and cribriform adenocarcinoma of minor salivary gland [10–17]. SDC is a high-grade salivary gland tumor, and its genetic changes are far more complex than those of previously mentioned low-grade salivary gland tumors. The most commonly observed somatic mutations in IC include *HER2* amplification, *AR* amplification, *HRAS* mutations, *PIK3CA* mutations, *AKT1* mutations, *TP53* mutations, and *NRAS* mutations [18–21]. Data on the genetic changes in IC are relatively limited due to its rarity. Recently, recurrent *RET* gene rearrangements have been found in individuals with IC, especially the intercalated duct type [5–7]. Weinreb et al. first reported *NCOA4-RET* fusion in one intercalated duct-type IC, and *RET* rearrangement was found in 47% of tested intercalated duct-type ICs but not in cases with apocrine features, which were found to harbor *PIK3CA* and/or *HRAS* hotspot mutations [5]. Later, Skálová et al. confirmed that the *NCOA4-RET* fusion was the major genetic change in intercalated duct-type IC, but they also discovered recurrent *TRIM27-RET* fusions in apocrine IC cases [6, 7]. *RET* rearrangement is now considered an important genetic change in IC.

IC and SDC of the salivary gland have morphologically similar counterparts in the breast. In the breast, it is well known that intraductal carcinoma is the precursor lesion of invasive carcinoma. However, the relationship between IC and SDC of the salivary gland is not clear as SDC is a high-grade and common malignancy, while IC is rare and usually low-grade in terms of histology. In this study, immunohistochemistry and mutation analysis (including *HRAS/PIK3CA* mutations, *RET* rearrangement, and *HER2* amplification) of IC (both intercalated duct type and apocrine type) and conventional SDC were performed and the results were compared. A rabbit monoclonal antibody against *NRAS* Q61R mutant protein has been found to cross-react with *HRAS* and *KRAS* Q61R mutant proteins in colorectal carcinoma and malignant melanoma [22, 23]. Immunohistochemistry of the antibody against *NRAS* (Q61R) was therefore performed in all cases to verify its value in the diagnosis of *HRAS* mutation in salivary gland tumors.

Methods

Case selection

Patients diagnosed with low-grade salivary duct carcinoma, IC, cribriform cystadenocarcinoma, cystadenocarcinoma, and salivary duct carcinoma between 1999 and 2018 in the

Department of Pathology at NTUH were evaluated by two pathologists (MSH and YJK). In this study, tumors were classified as IC when they show multiple cystically enlarged ducts and proliferation of ductal cells with appearance of mammary type atypical ductal hyperplasia or ductal carcinoma in situ. The small- to medium-sized tumor cells are bland-looking, with pale to eosinophilic cytoplasm and indistinct cell borders, having round to oval nuclei without prominent nucleoli and forming mixed cribriform, papillary-cystic, or filigreed patterns consistent with previous description [4–7]. ICs were further classified into (1) intercalated duct type when tumor cells had small eosinophilic to amphophilic cytoplasm, had cuboidal cells, and were positive for S100 immunohistochemistry and (2) apocrine type when tumor cells had granular eosinophilic cytoplasm, apocrine snouts, secretions, and were positive for *AR* immunohistochemistry [5–7]. Conventional SDC was confirmed using previous described criteria when ductal cells were high grade with architectural and cytological resemblances to in situ and invasive grade 2–3 ductal carcinoma of the breast featured by the presence of solid, cribriform, or comedonecrosis patterns, along with infiltrating ducts or nests [24]. A total of 4 intercalated duct-type ICs, 5 apocrine-type ICs, and 24 conventional SDCs were collected. SDC ex pleomorphic carcinoma was excluded from this study. Clinical data of these cases were obtained from their medical records. This study (201812013RINA) was approved by the Research Ethics Committee of National Taiwan University Hospital.

Immunohistochemistry

Immunohistochemistry was performed using an automated stainer (Ventana Benchmark; Roche Ventana, Tucson, AZ, USA). Tissue sections (thickness 4 μm) were dewaxed, rehydrated, and reacted with primary antibodies listed in Table 1. The *HER2* status was scored according to the American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update [25]. The *NRAS* Q61R staining was scored as 0 (negative), 1 (mild), 2 (moderate), and 3 (strong). The IHC results were evaluated by two pathologists (MSH and YHL).

Detection of *HRAS/PIK3CA* mutations

The *HRAS* and *PIK3CA* mutations were detected by PCR and Sanger sequencing. Regions of interest were macrodissected from unstained slides of the formalin-fixed, paraffin-embedded tissue and extracted using a DNeasy tissue kit (Qiagen, Hilden, Germany). PCR was performed using a HotStarTaq Master Mix kit (Qiagen) with the primers listed in Table 2. Successfully amplified products were purified and sequenced from both ends by DNA sequencing services using a BigDye Terminator kit (Applied Biosystems, Foster City, CA, USA) and an ABI Prism 3700 DNA Analyzer (Applied

Table 1 Antibodies used for immunohistochemistry

Antibody	Clone	Dilution	Antigen retrieval/time	Source
S100	15E2E2	RTU	w/o CC1	BioGenex
HER2	4B5	RTU	CC1/32 min	Ventana
Androgen receptor	SP107	RTU	CC1/32 min	Cell Marque
Mammaglobin	304-1A5 and 31A5	RTU	CC1/32 min	Zeta
P63	4A4	RTU	CC1/32 min	Ventana
SMA	1A4	RTU	CC1/8 min	Dako
SOX10	EP268	RTU	CC1/64 min	Bio SB
NRAS Q61R	RBT-NRAS	RTU	CC1/64 min	Bio SB

CC1 indicates EDTA buffer, pH 8.6

RTU ready to use, w/o without

Biosystems). All sequencing reactions were conducted in both forward and reverse directions, using amplicons from at least two independent PCRs. Specimens with mutations were confirmed in two rounds; only specimens that yielded the same result in both rounds were recorded as mutation positive.

FISH

All IC tissue samples were examined for *RET* break-apart fluorescent in situ hybridization (FISH) using ZytoLight SPEC RET Dual Color Break Apart Probe (ZytoVision, Bremerhaven, Germany) and ETV6 break-apart FISH (Abbott Molecular, USA) to rule out secretory carcinoma. FISH was performed as previously described [26]. Fifty non-overlapping tumor cell nuclei were evaluated in each patient. They were considered positive for target gene translocation when $\geq 20\%$ of the tumor nuclei exhibited a break-apart signal pattern. All patients were tested for *HER2* amplification using ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe (ZytoVision). Twenty nonoverlapping tumor cell nuclei were evaluated in each patient. *HER2* amplification was defined when the *ERBB2/CEN17* ratio is ≥ 2 or the average *HER2* copy number is ≥ 4 signals per cell [25].

Statistical analysis

Fisher's exact and Pearson's χ^2 tests were used to determine differences in categorical data. A two-sample Mann-Whitney *U* test was used to determine differences in age and size. Two-

sided $p < 0.05$ were considered statistically significant. The analysis for the prognostic effects including *HER2* mutation, T (T1 + T2 vs T3 + T4), N (N0 vs N1 + N2), age (< 65 vs ≥ 65), and sex on recurrence-free survival in the SDC group was evaluated by multivariate Cox regression analysis. SPSS 25.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses.

Results

Clinicopathological features of intercalated duct type and apocrine type IC

The main clinicopathological data of 9 IC patients are summarized in Table 3. This cohort comprises four female and six male patients, and the median age was 70 years (range from 44 to 85 years). The median tumor size was 3.3 cm (range from 0.8 to 6 cm). All tumors arose from the parotid gland except one located in the submandibular gland. Patients with intercalated duct type (44–70 years, median 58 years) were younger than those with apocrine type (50–85 years, median 80 years); the intercalated duct type also showed a female predominance. However, these differences did not reach statistical significance.

Microscopically, all nine IC patients showed variable-sized cysts lined by epithelial cells arranged in mixed micropapillary or filigreed epithelial tufts, cribriform, papillary, or solid patterns in variable proportions. Three intercalated duct-type ICs had poorly defined tumor borders and

Table 2 Primers for reverse transcription polymerase chain reaction (RT-PCR)

Target	Forward	Reverse	Size of PCR product (bp)
HRAS Exon3	5'-GTCCTCCTGCAGGAT TCCTA-3'	5'-CGGGGTTCCACCTGTACT-3'	179
PIK3CA Exon9	5'-AAAATATGACAAAGAAAGCTATATAAG-3'	5'-TCTCCATTTTAGCACTTACCTGTGA-3'	190
PIK3CA Exon20	5'-TTACTTATAGGTTTCAGGAGATGTGTT-3'	5'- TGTGTGGAAGATCCAATCCAT-3	258

Table 3 Clinicopathological features of intraductal carcinoma

No. type	Age	Sex	Site	Total size (cm)/ TNM	Immunohistochemistry					Mutation analysis					
					SOX10	AR	S100	HER2 (0–3+)	P63	Mammaglobin	NRAS (Q61R)	HRAS	PIK3CA	RET fusion	HER2 amplification
1	55	F	Parotid	0.8/T1sN0M0	(+)	(-)	(+)	0	(+)	(+)	0	(-)	(-)	(-)	(-)
2	44	M	Submandibular	1.2/T1sN0M0	(+)	(-)	(+)	0	(+)	(+)	0	(-)	(-)	(+)	(-)
3	61	F	Parotid	2.0/T1sNoM0	(+)	(-)	(+)	0	(+)	(+)	0	(-)	(-)	(+)	(-)
4	70	F	Parotid	6.0/T4aN2bM0 focal	(+)	(-)	(+)	0	(+)	(+)	0	(-)	(-)	(-)	(-)
5	72	M	Parotid	4.0/T2N0M0	(-)	(+)	(-)	1	(-)	(+)	3	Q61K	E545A	(-)	(-)
6	50	M	Parotid	4.5/T3N1M0	(-)	(+)	(-)	0	(-)	(+)	3	Q61R	H1047R	(-)	(-)
7	85	M	Parotid	3.3/T2N0M0	(-)	(+)	(-)	0	(+)	(+)	3	Q61R	H1047R	(-)	(-)
8	85	F	Parotid	2.5/T2N0M0	(-)	(+)	(-)	2	(+)	(+)	3	Q61R	H1047R	(-)	(-)
9	80	F	Parotid	4.0/T2N0M0	(-)	(+)	(-)	0	(+)	(+)	3	Q61R	E545K	(-)	(-)

F female, M male, IC intraductal carcinoma, HER2 human epidermal growth factor receptor 2

comprised multiple cysts with variable sizes, which were separated by a fibrous, nontumorous stroma (Fig. 1). One intercalated duct-type IC (no. 2) had MASC-like features with a relatively well-defined tumor border and a predominant papillary-cystic pattern (Fig. 1d). Hemorrhage and hemosiderin deposition were observed in three intercalated duct-type ICs. P63-positive myoepithelial cells were preserved in three and focally lost in one case.

The five apocrine-type cases comprised multiple, closely compacted cysts lined with tumor cells with eosinophilic cytoplasm and vesicular nuclei with occasionally prominent nucleoli and arranged in mixed cribriform, micropapillary, filigreed, or microcystic patterns (Fig. 2). All cases were relatively circumscribed without infiltrative growth. P63-positive myoepithelial cells were completely lost in two and mostly lost in three cases. Morphologically, apocrine-type ICs were similar to low-grade ductal carcinoma in situ (DCIS) or papillary carcinoma of the breast.

Immunohistochemically, the intercalated duct-type cases were positive for SOX10, S100, and mammaglobin and negative for AR, while the apocrine-type cases were negative for SOX10 and S100 and positive for AR and mammaglobin. All apocrine-type cases had strong NRAS (Q61R) staining (Fig. 2f), which correlated well with the HRAS mutation profile (3 with HRAS Q61R and 1 with Q61K mutation). Only two apocrine-type cases had mild to moderate HER2 staining.

Mutation analysis showed that two intercalated duct-type ICs had *RET* rearrangement. All apocrine-type IC cases had concurrent *HRAS/PIK3CA* mutations. All were negative for *HER2* amplification or *ETV6* rearrangement. During the clinical follow-up (10–96 months, median 72 months), none of the patients had tumor recurrence, distant metastasis, or tumor-related death.

Clinicopathological features of conventional SDC

The clinicopathological data of 24 patients with conventional SDC are listed in Table 4. This cohort comprises 22 male and 2 female patients with a median of 64.5 years. The median tumor size was 3.1 cm. Two-third of the cases were located in the parotid gland, and the others were located in the submandibular gland. All SDC showed high-grade cytological features with large nuclei, prominent nucleoli, coarse chromatin, abundant eosinophilic cytoplasm, frequent mitoses, and an infiltrating growth pattern with marked stromal desmoplastic change (Fig. 3). Immunohistochemically, the majority of SDC was positive for AR (91.7%) and all were negative for SOX10 and S100. HER2 IHC was 3+ in nine (37.5%), 2+ in seven (29.2%), and 0–1+ in eight (33.3%) patients. All nine HER2 IHC 3+ and two of seven HER2 IHC 2+ patients had *HER2* amplification (Fig. 3b). Three SDCs had strong (3+), and one had focal moderate (2+) staining for NRAS Q61R. Three SDCs with strong NRAS Q61R staining had HRAS Q61R

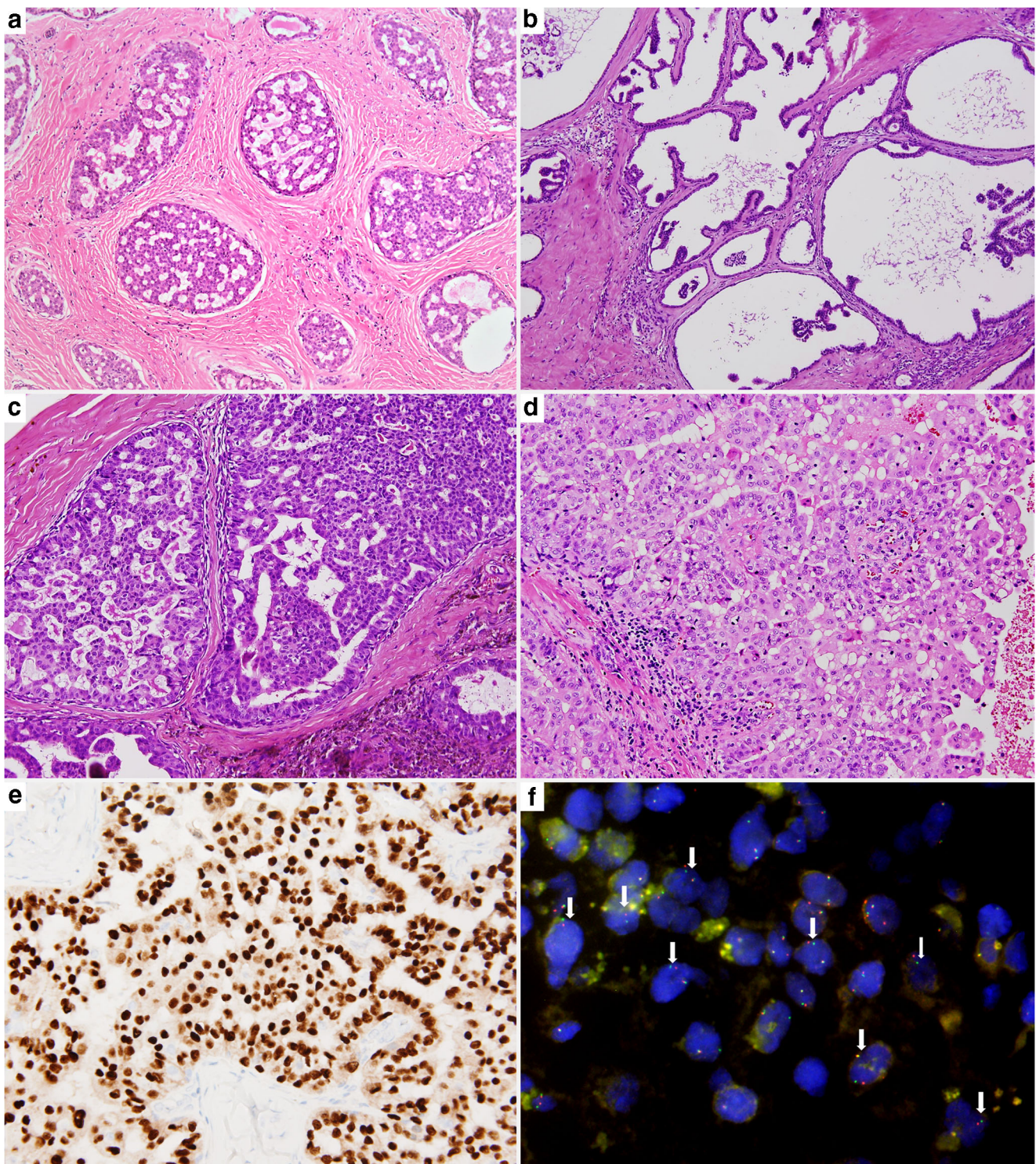


Fig. 1 **a** Intercalated duct-type IC typically presented as multiple cysts lined by cuboidal tumor cells with amphophilic cytoplasm arranged in lace-like structures morphologically similar to the usual ductal hyperplasia of the breast under low power examination (40 \times). **b** Other patterns including cystic, papillary, and micropapillary patterns were also common (100 \times). **c** Tumor cells had low- to intermediate-grade features with oval nuclei, inconspicuous nucleoli, and fine chromatin and arranged in tufts or papillary structures forming irregularly shaped secondary lumens

(200 \times). **d** One patient with intercalated duct-type IC had MASC-like features with eosinophilic cytoplasm; intracytoplasmic vacuoles; vesicular nuclei, arranged in a predominant cystic-papillary structure; hemorrhage; and hemosiderin deposition (200 \times). This patient had *RET* rearrangement and no *ETV6* translocation by fluorescence in situ hybridization (FISH). **e** Intercalated duct-type IC was diffusely positive for SOX10 (400 \times). **f** Two of four patients with intercalated duct-type IC had *RET* rearrangement confirmed by FISH. Arrows indicate split signals

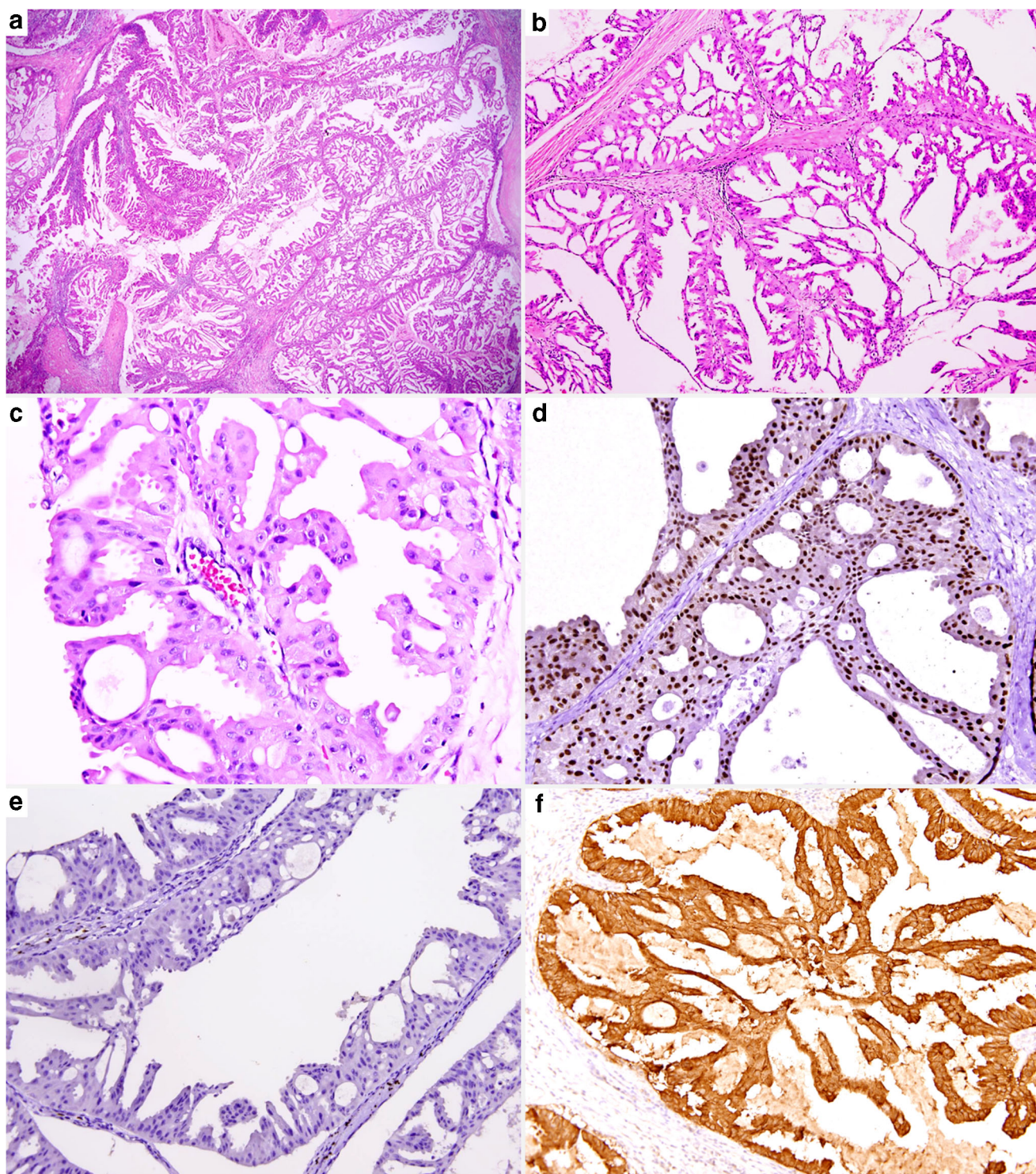


Fig. 2 Apocrine-type IC. **a** All cases comprised multiple cysts with relatively circumscribed tumor borders under low power field; tumor necrosis inside the cyst is not uncommon (40 \times). **b** These cysts were lined by tumor cells typically forming micropapillary, microcystic, cribriform, or filigree patterns (100 \times). **c** Tumor cells show low- to intermediate-grade features with eosinophilic cytoplasm, apical snouts,

apocrine snouts, and decapitation secretions (400 \times). **d** Patients with apocrine-type IC were positive for AR (200 \times), **e** negative for SOX10 (200 \times), and **f** positive for RAS Q61R, which were detected using NRAS Q61R antibody as a surrogate marker for HRAS Q61R mutation (200 \times)

mutation, the one with focal moderate (2+) staining had HRAS Q61K mutation, and those with negative or focal weak

staining were negative for *HRAS* mutation. All four SDCs with *HRAS* mutations harbored concurrent *PIK3CA*

Table 4 Clinicopathological features of conventional salivary duct carcinoma

No.	Age	Sex	Site	Size (cm)	TNM	Immunohistochemistry						Mutation analysis			
						SOX10	AR	S100	HER2 (0–3+)	P63	NRAS (Q61R)	HRAS	PIK3CA	RET fusion	HER2 amplification
1	79	M	Parotid	2.4	T4aN2bM0	(-)	(+)	(-)	3	(-)	0	(-)	(-)	(-)	(+)
2*	63	M	Submandibular	2.6	T2N0M0	(-)	(+)	(-)	3	(-)	0	(-)	(-)	(-)	(+)
3*	66	M	Submandibular	2.5	T2N2bM0	(-)	(+)	(-)	3	(-)	0	(-)	(-)	(-)	(+)
4	62	M	Submandibular	5.6	T4aN2bM0	(-)	(+)	(-)	3	(-)	0	(-)	(-)	(-)	(+)
5*	80	F	Parotid	3.8	T4aN2bM0	(-)	(+)	(-)	3	(-)	0	(-)	(-)	(-)	(+)
6	56	M	Parotid	3.7	T2N0M0	(-)	(+)	(-)	3	(-)	0	(-)	(-)	(-)	(+)
7*	62	M	Submandibular	2.5	T3N1M0	(-)	(+)	(-)	3	(-)	0	(-)	(-)	(-)	(+)
8	66	M	Submandibular	2.1	T2N0M0	(-)	(+)	(-)	3	(-) [#]	0	(-)	(-)	(-)	(+)
9*	90	M	Submandibular	3.5	T4N3M1	(-)	(+)	(-)	3	(-)	0	(-)	N/A	(-)	(+)
10	56	M	Submandibular	3.3	T2N2bM0	(-)	(+)	(-)	2	(-)	0–1	(-)	N/A	(-)	(+)
11	39	M	Parotid	4.7	T4aN1M0	(-)	(+)	(-)	2	(-)	0	(-)	N/A	(-)	(+)
12	86	M	Parotid	3.1	T4aN0M0	(-)	(+)	(-)	2	(-)	0	(-)	H1047R	(-)	(-)
13	37	M	Parotid	5.3	T4aN0M0	(-)	(+)	(-)	2	(-) [#]	3	Q61R	H1047R	(-)	(-)
14	71	M	Parotid	2.0	T3N2bM0	(-)	(+)	(-)	2	(-)	3	Q61R	H1047R	(-)	(-)
15	76	M	Parotid	3.1	T3N1M0	(-)	(+)	(-)	2	(-) [#]	1–2	Q61K	Q546K	(-)	(-)
16	56	M	Parotid	1.5	T1N1M0	(-)	(+)	(-)	2	(-)	3	Q61R	H1047R	(-)	(-)
17	82	M	Parotid	3.5	T2N0M0	(-)	(+)	(-)	1	(-) [#]	0	(-)	(-)	(-)	(-)
18	79	M	Parotid	3.5	T4aN1M0	(-)	(+)	(-)	0	(-)	0	(-)	(-)	(-)	(-)
19	60	M	Parotid	1.8	T1N2bM0	(-)	(+)	(-)	0	(-) [#]	0	(-)	(-)	(-)	(-)
20	79	M	Parotid	2	T1N0M0	(-)	(+)	(-)	0	(-)	0	(-)	(-)	(-)	(-)
21	62	M	Parotid	2.2	T2N2bM0	(-)	(+)	(-)	0	(-)	0	(-)	(-)	(-)	(-)
22	40	M	Parotid	3.5	T4aN0M0	(-)	(+)	(-)	0	(-)	0–1	(-)	N/A	(-)	(-)
23	71	M	Submandibular	3.4	T3N0M0	(-)	(-)	(-)	0	(-)	0–1	(-)	N/A	(-)	(-)
24	54	F	Parotid	3.0	T2N0M0	(-)	(-)	(-)	0	(-)	0	(-)	N/A	(-)	(-)

*These five cases had tumor recurrence, distant metastasis, and tumor-related death

[#]Intraductal carcinomatous component was present in these cases

mutations. During clinical follow-up (8 ~ 127 months, median 33 months), six cases (6/24, 25%) had tumor recurrence and five cases (5/24, 20.8%) had tumor-related death. HER2 amplification is the only factor with significant effect on tumor recurrence ($p = 0.047$) under multivariate analysis after adjusting T (T1 + T2 vs T3 + T4), N (N0 vs N+), age (< 65 vs ≥ 65), and sex. The small case number was inadequate for multivariate analysis on overall survival.

Comparison of IHC and mutation profiles between IC and conventional SDC

Results of clinicopathological comparison between IC and conventional SDC are summarized in Table 5. There is no significant difference in age, sex, location, tumor size, stage between intercalated duct-type and apocrine-type ICs. A significant male predominance and more advanced stage could be observed in the conventional SDC group. Immunohistochemically, intercalated

duct-type IC showed a distinct pattern different from apocrine-type IC and conventional SDC. The IHC expression of SOX10, S100, and mammaglobin in intercalated duct-type IC was similar to that of secretory carcinoma. The apocrine-type IC had an IHC expression more akin to that of conventional SDC, being negative for SOX10 and S100 and positive for AR. *RET* fusion was only present in intercalated duct-type IC. Concurrent *HRAS* and *PIK3CA* mutations were present in all patients with apocrine-type IC and a subset of conventional SDC (Fig. 3f). *HER2* amplification was the most common change found in conventional SDC (11/24, 45.8%).

Discussion

In this study, we reported nine patients with IC with low- to intermediate-grade nuclear features. Intercalated duct and apocrine-type IC had different morphological,

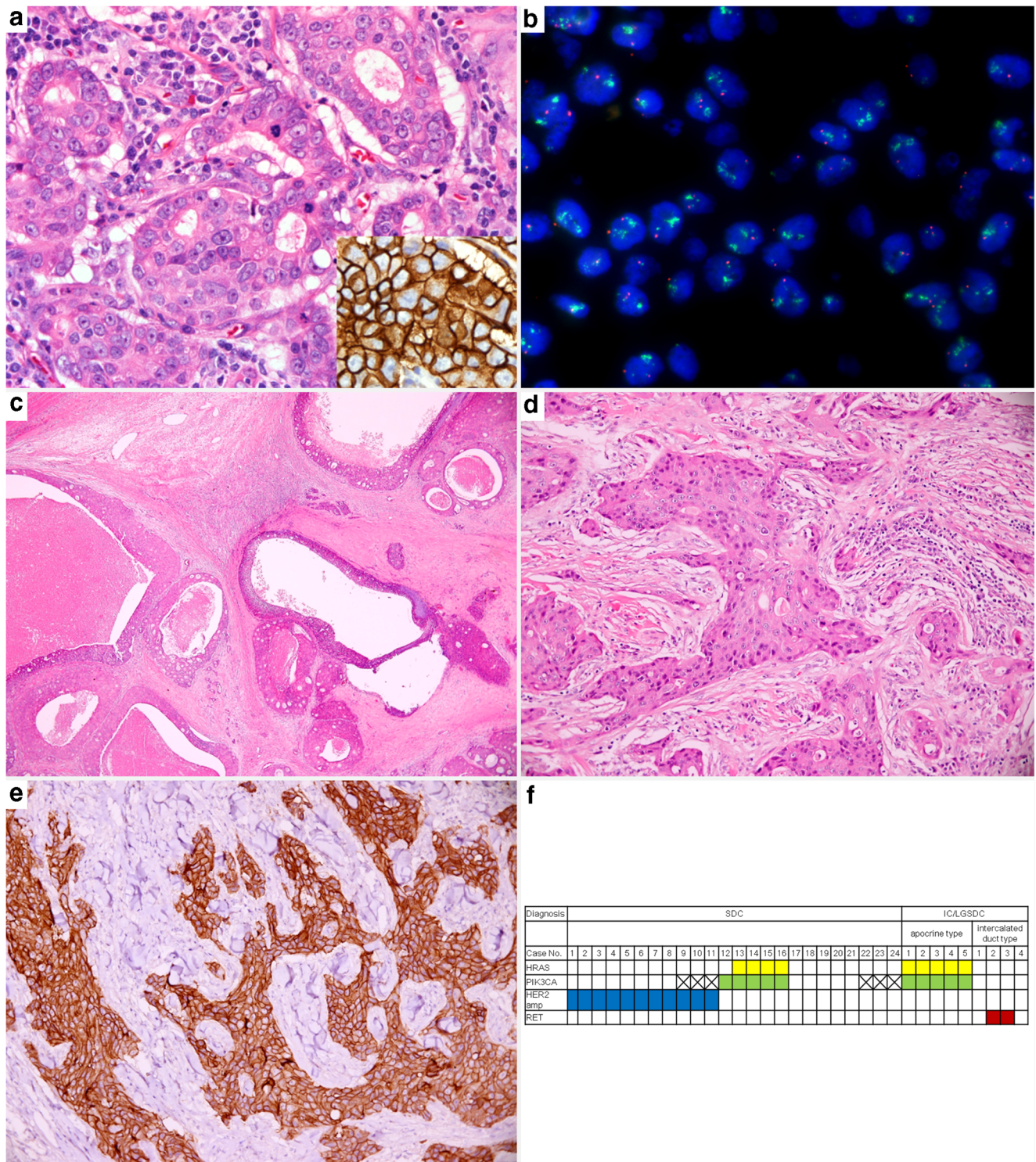


Fig. 3 **a** Conventional SDC showing infiltrating glands and high-grade cells with large nuclei, prominent nucleoli, eosinophilic cytoplasm, and strong staining for HER2 (400×), and **b** FISH revealed *HER2* amplification. **c** *HRAS/PIK3CA* mutated SDC with IC component morphologically similar to high-grade breast cancer (40×). **d** *HRAS/PIK3CA* mutated SDC

showing infiltrating nests and glands with marked stromal desmoplastic change and **e** diffusely positive for RAS Q61R using NRAS Q61R antibody as a surrogate marker for HRAS Q61R mutation (200×). **f** Mutation analysis of SDC and IC

immunohistochemical, and genetic changes. The four intercalated duct-type ICs comprised variable-sized cysts and most were loosely arranged in a fibrous background. Apocrine-type

ICs were circumscribed and composed of closely arranged cysts. According to the 2017 WHO, IC includes tumors previously diagnosed as low-grade salivary duct carcinoma or

Table 5 Results of clinicopathological comparison between intraductal carcinoma and salivary duct carcinoma

	Intercalated duct type IC (<i>n</i> = 4)	Apocrine type IC (<i>n</i> = 5)		Total IC (<i>n</i> = 9)	SDC (<i>n</i> = 24)	
Age (median)	44–70 (58)	50–85 (80)	<i>P</i> = 0.088	44–85 (70)	37–90 (64.5)	<i>P</i> = 0.817
Sex (M:F)	1:3	3:2	<i>P</i> = 0.524	4:5	22:2	<i>P</i> = 0.009
Location			<i>P</i> = 0.444			<i>P</i> = 0.384
- Parotid	3	5		8	16	
- Submandibular	1	0		1	8	
Size (cm) (median)	0.8–6 (1.6)	2.5–4.5 (4.0)	<i>P</i> = 0.410	0.8–6 (3.3)	1.5–5.6 (3.1)	<i>P</i> = 0.953
AJCC stage (8th edition)			<i>P</i> = 0.061			<i>P</i> = 0.007
0	3	0		3	0	
I	0	1		1	0	
II	0	3		3	6	
III	0	1		1	4	
IV	1	0		1	14	
IHC						
- SOX10	4/4, 100%	0			0	
- S100	4/4, 100%	0			0	
- AR	0	5/5, 100%			22/24, 91.7%	
- NRAS Q61R (2+/3+)	0	5/5, 100%			4/24, 16.7%	
Mutation analysis						
- RET fusion	2/4, 50%	0			0	
- HRAS	0	5/5, 100%			4/24, 16.7%	
- PIK3CA	0	5/5, 100%			5/18, 27.8%	
- HER2 amplification	0	0			11/24, 45.8%	
Died of disease	0	0			5/24, 20.8%	
Morphological counterpart in the breast	Usual ductal hyperplasia	Low-grade DCIS or papillary carcinoma			High-grade invasive ductal carcinoma (invasive carcinoma of no special type)	

IC intraductal carcinoma, SDC salivary duct carcinoma

p < 0.05 were considered statistically significant

low-grade cribriform cystadenocarcinoma as most of these tumors have preserve p63-positive rimming [1]. However, p63-positive rimming cells were absent focally in one intercalated duct-type IC and widely lost in all five apocrine-type ICs. Our finding is in conjunction with the recent study which reported the largest cohort of 33 ICs in the literature and 8 cases with focal or widespread invasive growth, especially those with apocrine features [7]. Therefore, the term “intraductal carcinoma,” which implies an in situ tumor, may not fully describe this type of tumor.

IC is the term used in 2017 WHO for the tumor originally described as low-grade salivary duct carcinoma or low-grade cribriform cystadenocarcinoma which is characterized by its low- to intermediate-grade cytological features [1]. Nevertheless, it also says IC can be further categorized as low, intermediate, and high grade types [1]. We think low- to

intermediate-grade IC properly represents low-grade salivary duct carcinoma or low-grade cribriform cystadenocarcinoma, whereas high-grade IC most likely represents the in situ component of conventional SDC. It may be inappropriate to use the same term “intraductal carcinoma” to describe these two different types of in situ neoplasms. Skálová et al. proposed to use “intercalated duct carcinoma, invasive or noninvasive” to replace “IC” in order to specify the tumor origin and make this entity more independent from SDC terminologically [7]. We concur their suggestion as that “intercalated duct carcinoma, invasive or non-invasive” is more precise and gives information of in situ or invasive status than other previously or currently used terms.

IC and SDC are generally considered as separate entities with different immunohistochemical profiles and driver mutations [5]. However, a subset of IC and SDC shares similar apocrine features [5–7, 9]. Apocrine IC was first described

by Weinreb et al. in 2006 as “low-grade intraductal carcinoma” with marked apocrine differentiation and diffusely expression of AR [9]. In 2018, Weinreb et al. further reported seven widely invasive, high-grade apocrine invasive adenocarcinomas morphologically similar to conventional SDC and one low- to intermediate-grade apocrine carcinoma with a predominant IC component [5]. In this study, we reported five apocrine type ICs characterized by low- to intermediate-grade nuclear features and relatively circumscribed borders. Unlike other studies showing *TRIM27-RET* fusion in some apocrine type IC [6, 26], all five apocrine type ICs in our study had concurrent *HRAS* and *PIK3CA* mutations but no *RET* rearrangement. Despite apocrine-type IC and conventional SDC share some pathological features (tumor necrosis, AR expression, and *HRAS/PIK3CA* mutations), the low- to intermediate-grade cytological features and lack of widely infiltrating growth in apocrine-type IC make it different from conventional SDC. Moreover, among all these cases, there is no morphological evidence of transition from apocrine IC to conventional SDC. We believe that low- to intermediate-grade apocrine IC and high-grade SDC represent two different types of tumors rather than a spectrum of differentiation. In the salivary gland, apocrine-type IC with circumscribed growth is akin to low-grade DCIS or papillary carcinoma of the breast, while SDC is similar to breast high-grade invasive ductal carcinoma.

SDC is a high-grade and aggressive salivary carcinoma resembling high-grade ductal carcinoma of the breast. Different histologic variants such as micropapillary, apocrine, mucinous, oncocytic, sarcomatoid, or rhabdoid types have been reported [21, 24, 27]. SDC typically has high-grade cytological features like large nuclei, coarse chromatin, prominent nucleoli, and frequent mitoses [24]. Comedonecrosis and stromal desmoplastic change are also not uncommon [24]. *HER2* amplification is the most common mutation in SDC with a frequency ranging from 15 to 40% [19–21, 24, 28]. Other somatic mutations that include *HRAS*, *PIK3CA*, *AKT1*, *TP53*, and *NRAS* have been reported [21]. SDC with *HER2* amplification could benefit from trastuzumab along with chemotherapy [29]. In this study, *HER2* amplification is the most common mutation (48.5%) followed by *PIK3CA* (27.8%) and *HRAS* (16.7%). Though *HER2* amplification is the only factor with significant effect on tumor recurrence under multivariate analysis in our cohort, other studies showed the number of lymph node metastases and facial nerve involvement are the most important prognostic factors for disease-free survival and overall survival in SDC [30, 31].

NRAS mutation is common in melanoma, thyroid follicular carcinoma, and a subset of colorectal carcinoma, and the Q61R is the most common *NRAS* mutation [32, 33]. SP174, a rabbit monoclonal antibody originally developed to detect *NRAS* Q61R-mutant protein, cross-reacts with *HRAS* and *KRAS* Q61R mutant proteins in colorectal carcinoma and

malignant melanoma [22, 23]. In this study, using another commercially available rabbit monoclonal antibody against *NRAS* Q61R (clone RBT-*NRAS*, for in vitro diagnostic use) to detect *HRAS* Q61R mutation yielded a high sensitivity (100%) and specificity. Seven patients with *HRAS* Q61R mutation showed diffuse and strong (3+) cytoplasmic staining, two with *HRAS* Q61K mutation showed at least focal moderate (2+) cytoplasmic staining, while the remaining 24 without *HRAS* mutation had negative (0) or very limited faint (0–1+) staining. This study demonstrated that another *NRAS* Q61R antibody (clone RBT-*NRAS*) can be used as a surrogate marker to detect *HRAS* Q61R mutation in the salivary gland tumors.

In conclusion, we reported nine ICs including four intercalated duct-type and five apocrine-type cases with different *SOX10*, *S100*, and *AR* staining patterns. All five apocrine-type ICs were morphologically mimicking breast low-grade DCIS or papillary carcinoma featured by their circumscribed borders and delicate fibrovascular stroma in arborizing pattern. *RET* fusion was detected in intercalated duct-type IC, concurrent *HRAS* and *PIK3CA* mutations were detected in apocrine IC, and *HER2* amplification was only observed in SDC. The monoclonal antibody (clone RBT-*NRAS*) against *NRAS* Q61R is a sensitive and specific surrogate marker for detecting *HRAS* Q61R mutation in salivary gland tumors.

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Author contributions MSH, YJT, and YJK designed the research study. MSH and YHL performed the research. MSH and YHL analyzed the data. MSH and YJK wrote the paper.

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Compliance with ethical standards

Clinical data of these cases were obtained from their medical records. This study (201812013RINA) was approved by the Research Ethics Committee of National Taiwan University Hospital.

Conflict of interest The authors declare that they have no conflict of interest.

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