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Oncocytic intraductal carcinoma of salivary glands: a distinct variant with *TRIM33–RET* fusions and *BRAF* V600E mutations

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Abstract

Aims: Salivary gland intraductal carcinoma (IDC) is a complex ductal neoplasm surrounded by a layer of myoepithelial cells. Recent insights have shown that there are three different types: intercalated duct-like, with frequent *NCOA4–RET* fusions; apocrine, with salivary duct carcinoma-like mutations; and mixed intercalated duct-like/apocrine, with *RET* fusions, including *TRIM27–RET*. In addition, an oncocytic IDC has been described, but it remains unclear whether it represents a fourth variant or simply oncocytic metaplasia of another IDC type. Our aim was to more completely characterize oncocytic IDC.

Methods and results: Six IDCs with oncocytic changes were retrieved from the authors' archives, from three men and three women ranging in age from 45 to 75 years (mean, 63 years).

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Author contributions

J. A. Bishop: conceived and designed analyses, collected data, performed analyses, and wrote the manuscript. M. Nakaguro: conceived and designed analyses, contributed data, and wrote the manuscript. R. D. Whaley: contributed data and wrote the manuscript. K. Ogura: contributed data. H. Imai: contributed data. I. Laklouk: contributed data. W. C. Faquin: contributed data and wrote the manuscript. P. M. Sadow: contributed data and wrote the manuscript. J. Gagan: performed analyses and wrote the manuscript. T. Nagao: conceived and designed analyses, collected data, performed analyses, and wrote the manuscript.

Conflict of interest

The authors state that they have no conflicts of interest.

Ethics approval and/or informed consent

Institutional review board approval was received (STU 112017–073). Informed content was not required for this study.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Five arose in the parotid gland, with one in an accessory parotid gland. Four patients with follow-up were free of disease after 1–23 months. Several immunostains (S100, mammaglobin, androgen receptor, and p63/p40) and molecular tools (RNA sequencing, *RET* fluorescence *in-situ* hybridisation, BRAF V600E VE1 immunohistochemistry, and Sanger sequencing) were applied. Histologically, the tumours were variably cystic with solid intracystic nodules often difficult to recognise as intraductal. In all, tumour ducts were positive for S100 and mammaglobin, negative for androgen receptor, and completely surrounded by myoepithelial cells positive for p63/p40. Molecular analysis revealed *TRIM33–RET* in two of six cases, *NCOA4–RET* in one of six cases, and *BRAF* V600E in two of six cases. One case had no identifiable alterations.

Conclusions: Oncocytic IDC shares similarities with intercalated duct-like IDC. Although additional verification is needed, the oncocytic variant appears to be sufficiently unique to be now regarded as the fourth distinct subtype of IDC. Because of its indolent nature, oncocytic IDC should be distinguished from histological mimics.

Keywords

BRAF V600E; intraductal carcinoma; *NCOA4–RET*; salivary gland neoplasms; *TRIM33–RET*

Introduction

Intraductal carcinoma (IDC) is an uncommon salivary gland neoplasm that has previously been referred to as ‘low-grade salivary duct carcinoma’ and ‘low-grade cribriform cystadenocarcinoma’.[1–5] Recent molecular analysis has shown that there are at least three distinctive IDC variants: (i) intercalated duct-like IDC, which is positive for S100 and SOX10 and often has *NCOA4–RET* fusion; (ii) apocrine IDC, which is negative for S100 and SOX10, is positive for androgen receptor, and has complex genetics, including *HRAS* and *PIK3CA* hotspot mutations; and (iii) hybrid or mixed IDC, which has both intercalated duct-like and apocrine features, and often harbours *RET* fusions, especially *TRIM27–RET*.[6–13] Apocrine IDC may be cytologically low-grade or high-grade, whereas the other two forms are usually low-grade. Regardless of this, when IDC is completely intraductal—i.e. surrounded by myoepithelial cells—it has an excellent prognosis, whereas widely invasive examples without myoepithelial cells are much more aggressive.[10–12,14]

In 2018, Nakaguro *et al*. described a form of IDC with prominent oncocytic features.[15] Oncocytic metaplasia is common in various salivary gland tumours, so it is currently unclear whether oncocytic IDC represents a variant of one of the well-established forms or a distinct tumour subtype. To answer this question, we performed molecular analyses on a series of oncocytic IDC cases.

Materials and methods

CASE SELECTION

With institutional review board approval (STU 112017-073), we identified cases of IDC with prominent oncocytic features from the authors’ consultation files. Three cases had been previously included in the original Nakaguro *et al*. series.[15] All cases were reviewed by two

or more of the authors, and were confirmed to meet the diagnostic criteria for IDC detailed in the 2017 World Health Organization classification of head and neck tumours.[5]

RNA SEQUENCING

Five of six cases were subjected to targeted RNA sequencing for fusions, as previously described.[16] Briefly, whole-slide tissue sections were cut at 10 μm, and Qiagen AllPrep kits (Qiagen, Germantown, MD, USA) were used for RNA isolation. A sequencing library was made by use of a modified TruSight RNA Pan-Cancer kit (Illumina, San Diego, CA, USA) with 1425 genes. Sequencing was performed on the Next-Seq 550 (Illumina) with a minimum of 6 000 000 mapped reads. Fusions were called by use of the Star-Fusion algorithm.[17] All fusions were manually reviewed via the Integrated Genomics Viewer (Broad Institute, Cambridge, MA, USA).

IMMUNOHISTOCHEMISTRY (IHC)

We performed IHC for S100 (Ventana Medical Systems, Tucson, AZ, USA), smooth muscle actin (Ventana), androgen receptor (Ventana), mammaglobin (Dako, Glostrup, Denmark), anti-mitochondria (Abcam, Cambridge, UK), anti-BRAF V600E VE1 (Ventana), and either p40 (BioCare Medical, Concord, CA, USA) or p63 (BioCare Medical). Staining was performed, with appropriate controls, on 4-μm whole-slide sections by the use of standardised automated protocols on Ventana BenchMark Ultra autostainers (Ventana).

FLUORESCENCE *IN-SITU* HYBRIDIZATION (FISH)

For five of six cases, we performed FISH for *RET* rearrangement according to the manufacturer's instructions, utilising a dual-colour DNA probe set (Agilent Technologies, Santa Clara, CA, USA) that hybridised to each end of *RET* in chromosome band 10q11.21. A total of 200 interphase nuclei within areas of tumour were manually enumerated, with separation of the 5′ and 3′ signals in >10% of cells considered to indicate positivity for *RET* rearrangement.

BRAF MUTATIONAL ANALYSIS

BRAF V600E mutations were detected with polymerase chain reaction (PCR) followed by Sanger sequencing. Briefly, DNA was extracted from unstained slides of the formalin-fixed paraffin-embedded (FFPE) tissue and extracted with a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). PCR products were purified with a QIAquick Spin Kit (Qiagen). Each purified product was directly sequenced by use of a forward primer with a BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3730 instrument (Applied Biosystems, Foster City, CA, USA). A mutation analysis was carried out to detect *BRAF* (exon 15), with the following primers: 5′-TCCTTTACTTACTACACCTCAGAT-3′ (BRAF-Exon15-F), and 5′-AGTGGAAAAATAGCCT-CAAT-3′ (BRAF-Exon15-R).

Results

Six oncocytic IDCs were identified. The clinical information and demographic information for these cases are summarised in Table 1. The tumours arose in three men and three women, ranging in age from 45 to 75 years (mean, 63 years). Each patient presented with a mass or

swelling in the parotid region, and underwent surgical resection. One patient also received external beam radiation because of a positive surgical margin. Five of the cases arose in the parotid gland, and one case arose in an accessory parotid gland. The tumours had an average size of 21 mm (range, 8–35 mm). Four patients with follow-up information available had not developed recurrence or metastasis after an average of 14.5 months (range, 1–23 months).

Histologically, five of six tumours had a prominent cystic tumour component. Whereas one case was entirely cystic and lined by micropapillary to flattened epithelium, the remaining four cystic cases had an intracystic solid nodule at low power (Figure 1A). The cysts were filled with eosinophilic secretions, and were often haemorrhagic. The remaining case was almost entirely solid and lobulated, lacking any macrocystic growth (Figure 1B). In the nodular tumour areas, the IDCs were characterised predominantly by compact collections of solid nodules, punctuated by scattered ductal spaces, and separated by thin strands of fibrosis (Figure 2A,B). Focal cribriform and/or papillary growth, typical of other forms of IDC, was also seen (Figure 2C,D). Pink intraluminal secretions were seen in each case; in one case they calcified, forming psammomatoid calcifications. Myoepithelial cells were difficult to discern on routine histology, especially around the compact, rounded nests in the more solid areas of the tumours. Oncocytic cellular features, defined as granular, eosinophilic cytoplasm and round nuclei with prominent nucleoli, were seen in all cases (Figure 3A). The extent of oncocytic change in the tumours ranged from 40% to 100% (mean, 85%). In the two cases that were not completely oncocytic, the non-oncocytic cellular component resembled intercalated ductal cells: small, cuboidal cells with minimal, pale cytoplasm, and small oval nuclei with open chromatin (Figure 3B). All of the IDCs were histologically low-grade, with no significant pleomorphism, low mitotic rates, and no necrosis. There were no irregular nests or desmoplasia to suggest overt stromal invasion.

The immunohistochemical findings are summarised in Table 2. The oncocytic IDCs were diffusely positive for S100 and mammaglobin (Figure 4A,B), and negative for androgen receptor. In all cases, the proliferative ducts were completely surrounded by a layer of myoepithelial cells that were positive for p63 and/or p40. These myoepithelial cells were present not only around the large cysts and small nests, but also in the back-to-back nests of the solid tumour components (Figure 4C,D). Anti-mitochondria staining was positive in four of four cases tested.

The molecular findings are summarised in Table 3. RNA sequencing identified a *TRIM33–RET* fusion in two cases (exon 11 of *TRIM33* and exon 12 of *RET*), with both showing a classic split signal on *RET* FISH. RNA sequencing also demonstrated *NCOA4–RET* fusion in one case; FISH was not performed in this case. RNA sequencing incidentally identified a probable *BRAF* V600E mutation in one of the fusion-negative cases, but because RNA sequencing is not the ideal method for detecting mutations, it was confirmed with IHC and Sanger sequencing (Figure 5). One additional oncocytic IDC with *BRAF* V600E mutation was subsequently found by the use of IHC and Sanger sequencing. Finally, one case—the one IDC that was highly cystic, with no solid growth—had no detectable genetic alterations. There were no obvious molecular–histological correlations with the identified genetic alterations.

To summarise the oncocytic IDC molecular results: two cases had *TRIM33–RET* fusions, two had *BRAF* V600E mutations, one had *NCOA4–RET* fusion, and one had no molecular alterations detected.

Discussion

In recent years, molecular testing has considerably altered our understanding of salivary gland tumours in general, and, in particular, the neoplasm known as IDC. These emerging studies have shown that IDC exists in at least three variants, each with unique histological, immunophenotypic and molecular features. Intercalated duct-type IDC is the most common. This variant is diffusely positive for S100 and SOX10, negative for androgen receptor, and has *NCOA4–RET* fusions in approximately half of reported cases.[10–12] Rarely, this variant may have alternative fusions like *STRN–ALK*, *TUT1–ETV5*, and *KIAA1217–RET*, and some cases have been fusion-negative.[10,18] The apocrine form of IDC is negative for S100 and SOX10 but strongly positive for androgen receptor. Apocrine IDC has not been found to harbour fusions; instead this variant has a complex mutational profile (e.g. *PIK3CA* and *HRAS* mutations, *TP53* loss) reminiscent of salivary duct carcinoma.[6,8,11,12] The mixed intercalated duct/apocrine form of IDC has hybrid histological and immunophenotypic features of the other IDC types. Like intercalated duct-like IDC, mixed IDC commonly harbours *RET* fusions, although *TRIM27–RET* is more common than *NCOA4–RET* in this variant.[9–11] More recently, it has been shown that the myoepithelial cells are probably neoplastic in fusion-positive IDCs, raising interesting questions about the staging and terminology for this enigmatic tumour.[19] Indeed, the term ‘intraductal’ may be inaccurate and could be abandoned in future classification schemes. Importantly, all forms of IDC are very indolent when they are low-grade and contain myoepithelial cells.

In 2018, Nakaguro *et al.* reported a form of low-grade IDC with oncocytic features.[15] Although these authors believed that it represented a unique form of IDC, at the time it was difficult to exclude the possibility of oncocytic metaplastic change in intercalated duct-like IDCs.[15] After all, oncocytic metaplasia can be seen in almost all salivary gland tumour types, perhaps most notably in the oncocytic variant of mucoepidermoid carcinoma.[20,21] In most salivary gland tumours, oncocytic variants are genetically identical to their non-oncocytic counterparts, so we performed genetic analysis to determine whether oncocytic IDC is molecularly distinct from the other three forms of this tumour.

We found that oncocytic IDC has some features that are reminiscent of intercalated duct-type IDC. Both types of IDC predominate in the parotid gland of adults and show indolent behaviour, and they have almost identical immunophenotypes. Moreover, our genetic analysis showed that *RET* fusions were present in half of the oncocytic IDCs, which is similar to the rate reported for intercalated duct-type IDC, and one case harboured the *NCOA4–RET* fusion, which is most often seen in that more common variant. The fact that two of six cases had a component of typical intercalated duct-like morphology also supports the notion that these are related tumours.

On the other hand, we also demonstrated that oncocytic IDC is unique in many respects. Aside from the obvious distinctively oncocytic cytomorphology, the compact, solid growth

seen in most cases is also unusual. This growth, especially when it is not accompanied by a cystic component, can make it difficult to recognise the intraductal nature of oncocytic IDC. Also, *TRIM33–RET* is a fusion that has not been previously reported in IDC or any other salivary gland tumour. The fact that it was found in two of six oncocytic IDCs points to the uniqueness of this variant. This relationship is similar to that seen with *TRIM27–RET*, which is not always found in mixed intercalated duct/apocrine IDCs (occasional cases have *NCOA4–RET*), but does appear to be specific for that variant. Finally, the *BRAF* V600E mutation, which was also identified in two oncocytic IDCs, has also never previously been reported in IDCs. Table 4 summarises an updated classification of IDC.

The finding of two oncocytic IDCs with *TRIM33–RET* expands the list of salivary gland tumours shown to have *RET* fusions, which includes not only the intercalated duct and hybrid forms of IDC, but also occasional secretory carcinomas and, possibly, rare salivary duct carcinomas.[7,22–24] *TRIM33* is located on chromosome 1p13.2. Like the gene product of *TRIM27*, TRIM33 is a member of the tripartite motif-containing (TRIM) family of proteins with E3 ubiquitin ligase activity, which are involved in numerous crucial biological processes.[25] Although *TRIM33–RET* has never been reported in salivary gland tumours, it has rarely been described as a presumed driver in papillary thyroid carcinoma and lung adenocarcinoma.[26,27] We found that *TRIM33–RET* is positive on break-apart *RET* FISH, like *TRIM27–RET* but unlike *NCOA4–RET* (a subtle inversion that is difficult to visualise), which simplifies testing, as *RET* FISH is more readily available than next-generation sequencing.

Although they have not previously been seen in IDC, *BRAF* V600E mutations have been described in salivary gland tumours. Rare cases of salivary duct carcinoma have shown this mutation.[28–30] Although this link is intriguing, the lack of high-grade features or androgen receptor positivity in oncocytic IDC makes it unlikely that this tumour is related to salivary duct carcinoma. A rare but distinctive salivary duct tumour known as sialadenoma papilliferum harbours the *BRAF* V600E mutation in approximately 70–75% of cases.[31,32] Like IDC, sialadenoma papilliferum is an indolent, low-grade ductal proliferation that is surrounded by a layer of basal cells. On the other hand, sialadenoma papilliferum almost always occurs in the oral cavity, and has a component of papillary or verrucoid squamous proliferation that is lacking in IDC. Moreover, most sialadenoma papilliferum cases are not oncocytic. Although there is an oncocytic form of sialadenoma papilliferum, this variant, surprisingly, does not actually harbour the *BRAF* V600E mutation.[3] Accordingly, although sialadenoma papilliferum and oncocytic IDC may well belong to a family of low-grade intraductal *BRAF*-mutated neoplasms, they are distinct from each other.

Although it is not entirely clear whether IDC is a form of carcinoma *in situ* or has a pushing invasive front, what is evident is that, in their pure form (i.e. with myoepithelial cells and without frank stromal invasion), all variants of IDC are very indolent tumours. Accordingly, they should be distinguished from other salivary gland carcinomas that have an increased capacity for aggressive behaviour. The prominent solid growth in most IDCs may make it difficult to recognise, and more likely to be confused with histological mimics. Secretory carcinoma, in particular, may resemble IDC, with its oncocytic appearance, mixed cystic, papillary and solid growth patterns, and consistent S100 and mammaglobin

positivity. Recognising focal areas of more typical IDC growth (isolated, cribriform, or micropapillary nests) is helpful. In addition, although secretory carcinoma may have very focal intraductal growth, it lacks the diffuse network of myoepithelial cells seen in IDC. Another potential pitfall is mistaking oncocytic IDC for epithelial–myoepithelial carcinoma, especially the oncocytic/apocrine variant. Again, identifying typical IDC foci is helpful. Although both tumours have a myoepithelial cell component, the myoepithelial cells of epithelial–myoepithelial carcinoma tend to be much larger and more prominent, and they often have a clear cell appearance. Finally, both secretory carcinoma (*ETV6* fusions) and epithelial–myoepithelial carcinoma (frequent *HRAS* mutations), which are tumours with some morphological and architectural overlap with oncocytic IDC, appear to have discernible genetic differences from IDC.[33,34]

In summary, oncocytic IDC is a variant of IDC that has unique histological and immunophenotypic features. It may show prominent solid growth and variable genetic alterations (*TRIM33–RET* and *BRAF* V600E) that have not previously been described in IDC. As with other salivary tumours, a single distinct molecular profile is not seen. However, much like other reported IDC variants, oncocytic IDC appears to be an indolent tumour. Given the heterogeneous molecular findings and small number of cases, additional cases will be needed for confirmation of these findings.

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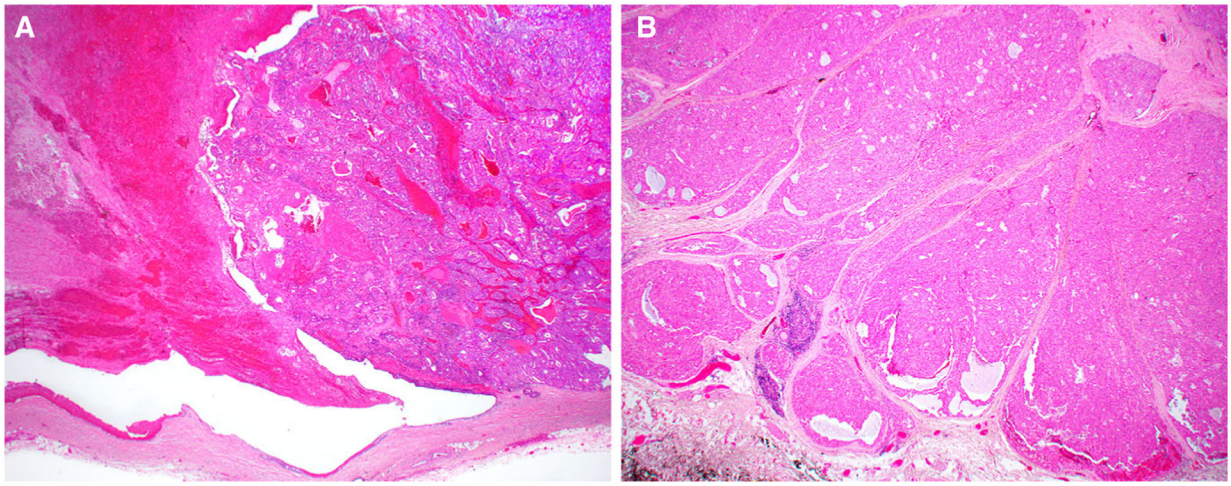

Figure 1.

Most oncocytic intraductal carcinomas (case 2 is shown) were partly macrocystic with intracystic secretions and haemorrhage (left), and intracystic solid tumour nodules (right) (**A**). Case 4, however, had no significant cystic growth and instead appeared as a solid, nodular tumour (**B**).

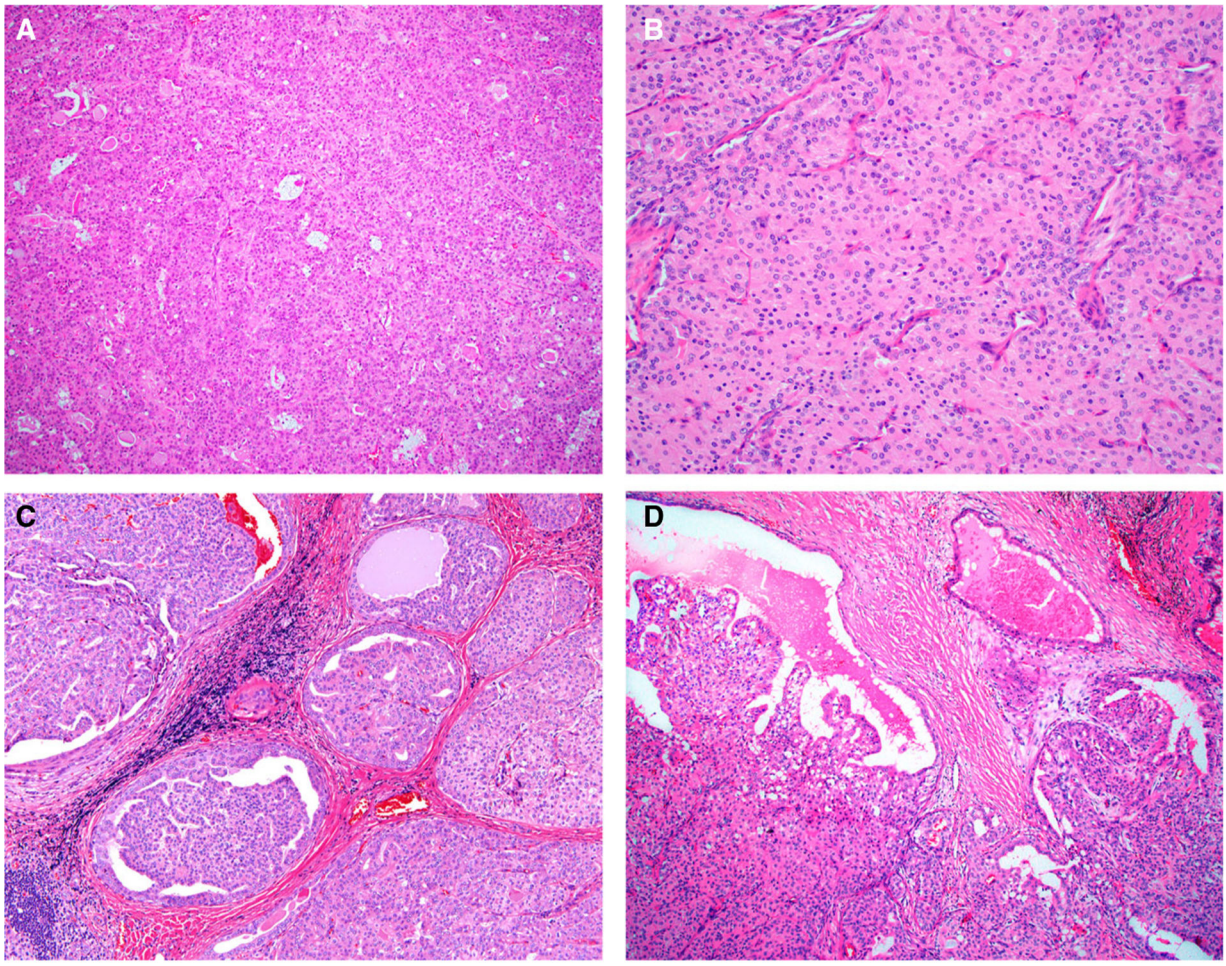

Figure 2.

The solid areas of oncocytic intraductal carcinoma (IDC) consisted of back-to-back solid nodules, punctuated by scattered ducts (cases 4 and 1 are shown) (**A, B**). Most cases also showed areas that were more typical of IDC, with cribriform nodules (case 4 is shown) (**C**) and papillary cystic growth (case 1 is shown) (**D**).

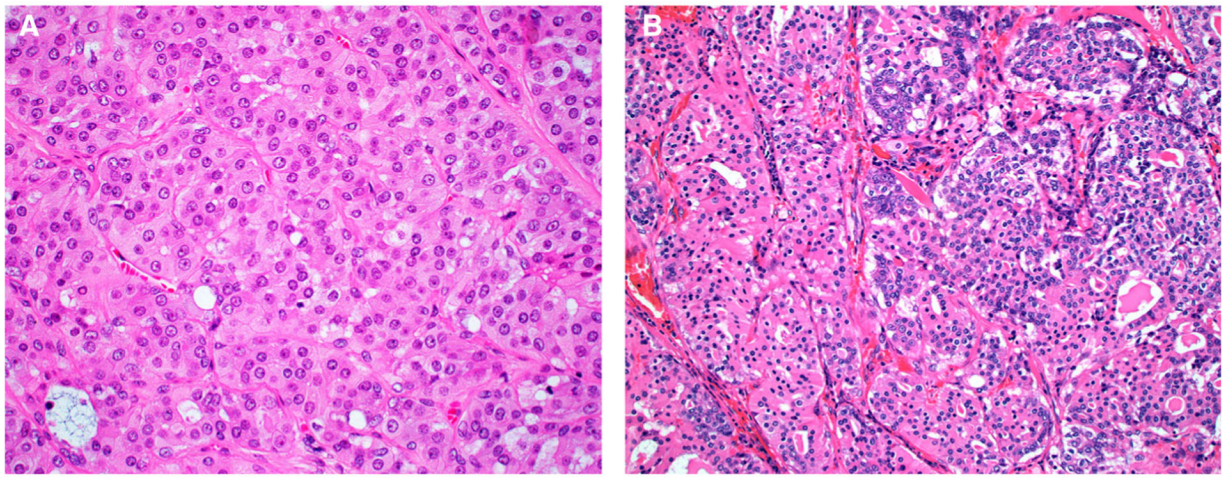

Figure 3.

Oncocytes in the oncocytic intraductal carcinomas had abundant, eosinophilic, granular cytoplasm, with round nuclei that have prominent nucleoli (case 4 is shown) (**A**). Two cases had mixed oncocytic (left) and intercalated duct-like (right) cellular features. The intercalated duct-like cells had a more basophilic appearance, with less cytoplasm and more oval, pale nuclei (case 2 is shown) (**B**).

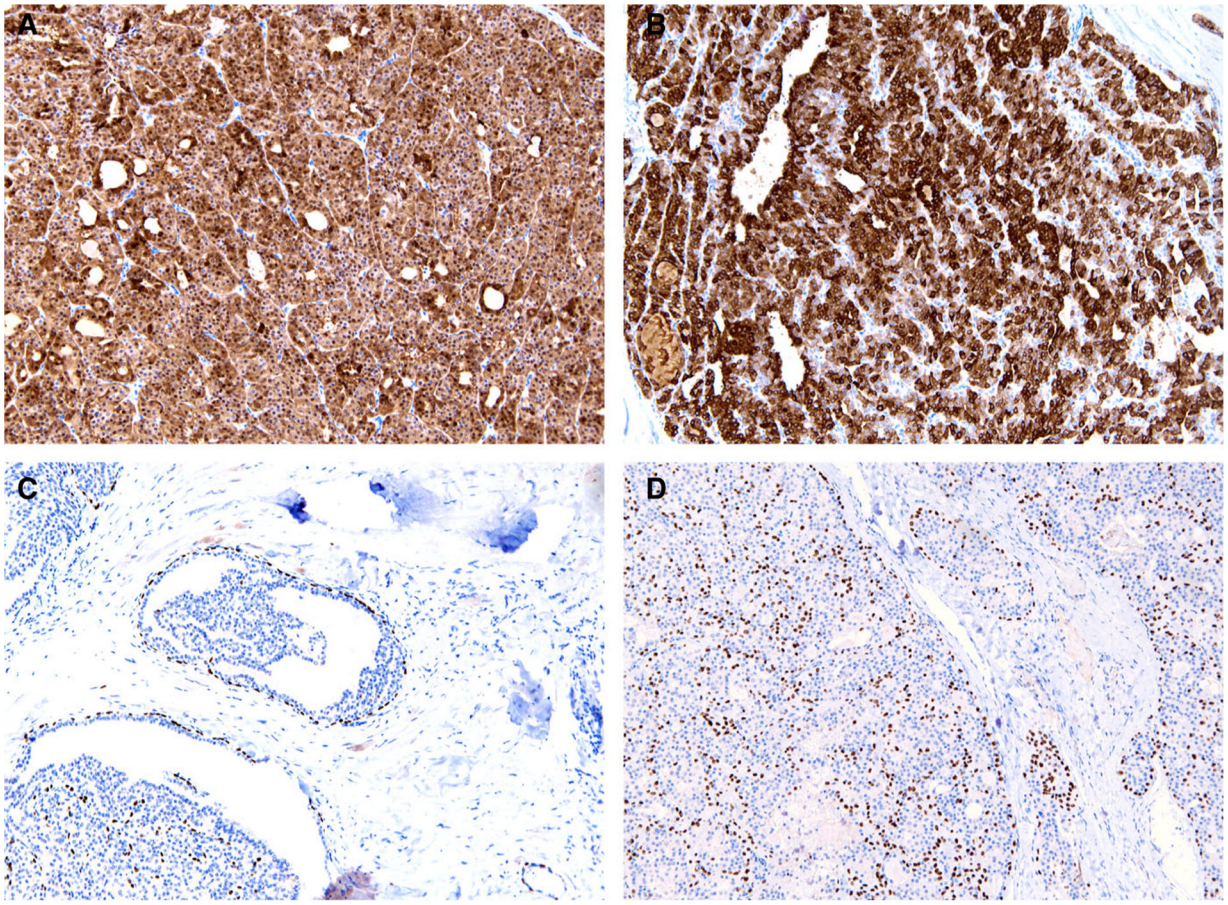

Figure 4.

Oncocytic intraductal carcinoma was diffusely positive for S100 (**A**) and mammaglobin (**B**). The myoepithelial marker p63 was positive around cysts and cribriform nests (**C**) and also within the solid tumour components (**D**). Case 4 is shown.

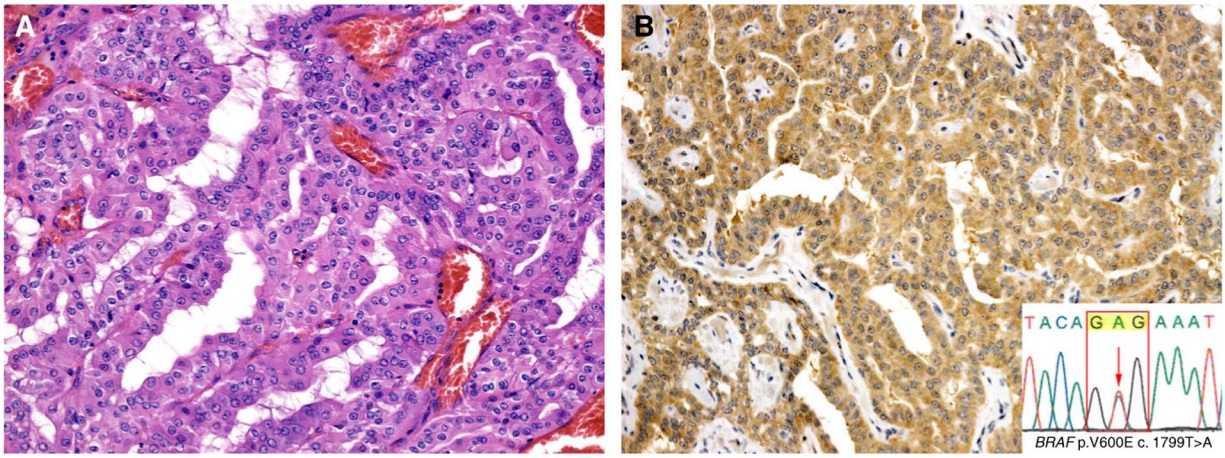

Figure 5.

Two cases of oncocytic intraductal carcinoma (**A**) were positive for anti-BRAF V600E VE1 immunohistochemistry (**B**), with mutations confirmed by Sanger sequencing (inset). Case 6 is shown.

Table 1.

Clinical and demographic information

Case	Age (years)	Sex	Site	Size (mm)	Follow-up	Reference
1	74	M	Right parotid gland	35	NED at 23 months following surgery	Nakaguro *et al.*[15]
2	45	M	Left accessory parotid gland	33	NED at 23 months following surgery	Nakaguro *et al.*[15]
3	63	M	Right parotid gland	8	NA	None
4	50	F	Right parotid gland	18	NED at 1 month following surgery, plan for radiotherapy because of positive margins	None
5	69	F	Right parotid gland	20	NA	None
6	75	F	Right parotid gland	12	NED at 11 months following surgery	Nakaguro *et al.*[15]

F, female; M, male; NA, not available; NED, no evidence of disease.

Table 2.

Immunohistochemical testing

Case	S100	Mammaglobin	p63/p40	Androgen receptor	Mitochondria	Molecular alteration
1	Diffuse	Diffuse	Peripheral	Negative	Positive	*BRAF* V600E mutation
2	Diffuse	Diffuse	Peripheral	Negative	Positive	*TRIM33–RET* fusion
3	Diffuse	Diffuse	Peripheral	Negative	Positive	None found
4	Diffuse	Diffuse	Peripheral	Negative	NA	*TRIM33–RET* fusion
5	Diffuse	Diffuse	Peripheral	Negative	NA	*NCOA4–RET* fusion
6	Diffuse	Diffuse	Peripheral	Negative	Positive	*BRAF* V600E mutation

NA, not available.

Table 3.

Molecular analysis

Case	Summary of histological findings	RNA sequencing	*RET* FISH	*BRAF* V600E IHC	*BRAF* V600E Sanger sequencing	Summary of molecular results
1	100% oncocytic; 50% cystic, with solid, cribriform and papillary patterns	No fusions, probable *BRAF* V600E mutation	Negative	Positive	Positive	*BRAF* V600E mutation
2	40% oncocytic; 60% cystic, with solid, cribriform and tubular patterns	*TRIM33–RET* fusion	Positive, classic split	ND	ND	*TRIM33–RET* fusion
3	70% oncocytic; 100% cystic, lined by flattened or micropapillary patterns	No fusions	Negative	ND	ND	None found
4	100% oncocytic; solid and cribriform patterns, not cystic	*TRIM33–RET* fusion	Positive, classic split	ND	ND	*TRIM33–RET* fusion
5	100% oncocytic; 70% cystic with cribriform and tubular patterns	*NCOA4–RET* fusion	ND	ND	ND	*NCOA4–RET* fusion
6	100% oncocytic; 80% cystic with solid and papillary patterns	ND	Negative	Positive	Positive	*BRAF* V600E mutation

FISH, fluorescence *in-situ* hybridisation; IHC, immunohistochemistry; ND, not done.

Table 4.

Current classification of salivary gland intraductal carcinoma

Variant	Frequency	Grade	S100	Androgen receptor	Genetics	Associated invasion
Intercalated duct	Most common	Low	++	−	Usually *NCOA4–RET* Rare *STRN–ALK*, *TUT1–ETV5*, and *KIAA1217–RET*	Rare
Apocrine	Uncommon	Low or high	−	++	Complex, with multiple mutations (*HRAS*, *PIK3CA*, and others) No fusions	Common
Mixed intercalated duct/apocrine	Rare	Low	++ in intercalated duct-like areas	++ in apocrine areas	Usually *TRIM27–RET* Occasionally *NCOA4–RET*	Rare
Oncocytic	Rare	Low	++	−	*TRIM33–RET* *BRAF* V600E mutation Occasionally *NCOA4–RET*	Not reported