Hybrid Carcinoma of The Parotid Gland: A Report of Three Extremely Rare Cases with An Immunohistochemical Analysis and A Review of The Literature

Kimihide Kusafuka (k-kusafuka@i.shizuoka-pho.jp)
Shizuoka General Hospital https://orcid.org/0000-0003-3482-7447
Masaru Yamashita
Kagoshima University: Kagoshima Daigaku
Shoji Yamanaka
Yokohama City University Urafane Hospital: Yokohama Shiritsu Daigaku Fuzoku Shimin Sogo Iryo Center
Kazuki HIrata
Shizuoka General Hospital
Aki Kubota
Shizuoka General Hospital: Shizuoka Kenritsu Sogo Byoin
Akinori Murakami
Shizuoka General Hospital: Shizuoka Kenritsu Sogo Byoin
Aya Muramatsu
Shizuoka General Hospital: Shizuoka Kenritsu Sogo Byoin
Kazumori Arai
Shizuoka General Hospital: Shizuoka Kenritsu Sogo Byoin
Makoto Suzuki
Shizuoka General Hospital: Shizuoka Kenritsu Sogo Byoin

Case Report

Keywords: hybrid carcinoma, salivary duct carcinoma, squamous cell carcinoma, large cell neuroendocrine carcinoma, epithelial-myoepithelial carcinoma

Posted Date: September 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-74800/v1

Version of Record: A version of this preprint was published at International Journal of Surgical Pathology on September 6th, 2021. See the published version at https://doi.org/10.1177/10668969211036942.

Abstract

Background: Hybrid carcinoma (HC) of the salivary glands is defined as when two or more kinds of carcinoma exist at the same location in a single mass. Herein, we report 3 rare cases of salivary gland HC.

Case Presentation: Case 1 involved an 86-year-old Japanese male. Case 2 involved a 70-year-old Japanese female. Case 3 involved a 66-year-old Japanese male. Histologically, case 1 involved a combination of salivary duct carcinoma (SDC) and squamous cell carcinoma (SqCC). Immunostaining indicated that the former was positive for gross cystic disease fluid protein (GCDFP)-15 and androgen receptor (AR), whereas the latter expressed cytokeratin (CK) 5/6 and p63. Case 2 involved a combination of SqCC and neuroendocrine carcinoma (NEC). Immunostaining indicated that the former was positive for CK5/6 and p40, whereas the latter was positive for synaptophysin and neural cell adhesion molecule. Case 3 involved a combination of SDC and epithelial-myoepithelial carcinoma (EMC). Immunostaining indicated that the former was positive for GCDFP-15 and AR, whereas the inner cells of the latter were positive for CK7, and the outer cells of the latter were positive for alpha-smooth muscle actin and p40. A transitional zone between the SDC and EMC existed in case 3.

Conclusions: Therefore, we diagnosed them as parotid gland HC. This is the first report of a case of HC involving a combination of NEC and SqCC. In case 3, it was speculated that high-grade SDC arose from low-grade EMC. However, as HC is different from carcinoma with high-grade transformation, HC should be diagnosed carefully.

Introduction

In 1996, Seifert and Donath described a series of 5 cases of salivary gland tumors containing two different types of neoplasms and called them “hybrid tumors”[1]. Nowadays, hybrid tumors are defined as tumors consisting of at least two different tumor entities that develop and merge in the same topological area. Hybrid tumors account for up to 0.1% of all salivary gland tumors, and both benign and malignant hybrid tumors have been reported [1, 2].

More than 30 cases of HC have been reported in the English literature (Table 1) [1, 3–22]. However, only 2 cases involving a combination of SDC and squamous cell carcinoma (SqCC) [3] and 3 cases involving a combination of salivary duct carcinoma (SDC) and epithelial-myoepithelial carcinoma (EMC) have been reported [5, 16, 23]. To the best of our knowledge, it is the first report about a case of HC involving SqCC and large cell neuroendocrine carcinoma (LCNEC). We report 3 additional cases of extremely rare HC, together with their immunohistochemical findings, and review the previous HC cases reported in the English literature.

Case Presentation

We extracted 3 cases of salivary gland HC from a pathology file and one of the author’s consultation files (K.K.) for the period from 1992–2019. All cases involved primary salivary gland HC. All of the slides for these cases were reevaluated, with particular attention paid to the two different histological components, the presence or absence of transitional zones, and the absence of primary neoplasms from other organs, especially in the head and neck region.

Case 1

Clinical findings

The patient was an 85-year-old Japanese male, who had noticed a rapidly swelling mass in the left parotid region some years ago. He was admitted to his local clinic and was diagnosed with adenocarcinoma after a fine-needle aspiration biopsy (FNAB). He was admitted to our hospital 20 months later. An immovable mass was present in the left parotid region, but the patient was not suffering any pain or facial nerve paralysis. Magnetic resonance imaging (MRI) showed an ill-defined high intensity region and a focal low intensity region on T1-weighted imaging, and a low intensity region and focal high intensity region on T2-weighted imaging (Figure 1A). Positron emission tomography (PET) showed accumulation in the left parotid gland mass. Under a clinical diagnosis of left parotid gland cancer (pT4aN0M0), total parotidectomy with skin and facial nerve resection was performed. No postoperative treatment was administered. There was no evidence of local recurrence or metastasis at 2 years after the operation.

Pathological findings

Macroscopically, an ill-defined yellowish-white mass, with partial cystic changes, and yellowish-green contents inside the cystic spaces, was seen (Figure 1B). The tumor measured 4.0 x 3.2 x 5.3 cm.

Histologically, the tumor was composed of a combination of an SDC and an SqCC. The major component (i.e., the SDC), which accounted for 80% of the tumor, had a classical and typical histology, involving microcystic to marked cystic dilation, and exhibited Roman-bridge structures and comedonecrosis by cancer cells (Figure 1C), together with eosinophilic cytoplasm, marked nuclear pleomorphism, and a high mitotic rate. Immunohistochemical staining was performed on 4-μm-thick sections, which were cut from formalin-fixed and paraffin-embedded tissue. The tumors were considered to be diffusely positive (+++), positive (++), partially positive (+), and focally positive (F+) when ≥50%, 20–49%, 10–19%, and 1–9% of the neoplastic cells were positive, respectively. The HER2 score was estimated according to the criteria of Wolff et al [24]. Epidermal growth factor receptor (EGFR) expression was categorized as positive or negative. According to Boyle's evaluation criteria [25], a tumor was considered to be p53-positive if >50% of the tumor cell nuclei showed strong reactivity, which was considered to be indicative of a *p53* point mutation. Carcinoma cells that exhibited no p53 immunostaining despite weak positivity being seen in the infiltrating lymphocytes raised a high suspicion of the deletion (i.e., loss) of *p53*. Weak of sparse p53 immunostaining was considered to be indicative of the presence of wild-type *p53*. The antibodies used in this study and the results of the immunohistochemical analysis are summarized in Table 2. Immunostaining showed that the SDC component was strongly positive for cytokeratin (CK) 7, gross cystic disease fluid protein (GCDFP)-15, and androgen receptor (AR), and partially positive for EGFR and CK5/6 (Figures 1D), but negative for human epidermal growth factor receptor 2 (HER2), p63, and p40. Therefore, the SDC was subclassified into the apocrine B phenotype, according to Takase's classification of SDC [26].

At least 20% of the tumor was made up of nest-like structures composed of proliferating cancer cells, which exhibited keratinization and squamous differentiation (Figure 1E). The cancer cells in this component displayed marked nuclear atypia, single cell keratinization, and intercellular bridges. This component was considered to be a moderately differentiated SqCC. Immunostaining indicated that this component was diffusely positive for CK5/6 (Figure 1F), p63, p40, and EGFR, and focally positive for CK7, but entirely negative for GCDFP-15, AR, and HER2, which are SDC markers.

As both components were focally and weakly positive for p53, they were considered to exhibit the wild-type pattern. The Ki-67 indices of the SDC and SqCC components were 58% and 43%, respectively. Both components were well demarcated, but some areas showed mixed nests composed of both SDC and SqCC.

We finally diagnosed the patient with HC composed of SDC and SqCC.

Case 2

Clinical findings

The patient was a 79-year-old Japanese female, who suffered from swelling of the left parotid region and a disturbance of oral opening. She noticed a small painless mass in the left parotid region. The mass rapidly enlarged, and she was admitted to another hospital a month later. An immovable mass was seen in the left parotid region, but no lymph node swelling or facial nerve paralysis was observed. FNAB and MRI showed no malignancy at that time. After approximately 3 months, FNAB was performed again, and the lesion was cytologically diagnosed as SqCC. Computed tomography (CT) revealed an ill-defined irregularly shaped mass in the left parotid gland, and MRI showed a region of iso-intensity on T1-weighted imaging and low intensity on T2-weighted imaging. PET only showed accumulation in the left parotid mass and did not show any other primary lesion. Under a clinical diagnosis of left parotid gland cancer (cT4aN0M0), left total parotidectomy and left mandibulectomy were performed. Postoperative radiotherapy (66 Gy) was administered. There was no evidence of recurrence or metastasis at 7 months after the operation.

Pathological findings

Macroscopically, a single mass was seen (Figure 2A). An examination of the cut surface of the tumor revealed an ill-defined, yellowish-white mass. The central portion of the mass was somewhat whitish.

Histologically, the major component, which accounted for approximately 65% of the tumor, was an SqCC. This component showed marked keratinization, squamous differentiation, and intercellular bridges (Figure 2B). Immunostaining indicated that the cancer cells were diffusely positive for CK5/6, p63, and p40 (Supplemental figure 2) and partially positive for CK8, but negative for neural cell adhesion molecule (NCAM), chromogranin-A, synaptophysin, insulinoma-associated protein (INSM)1, and CK20.

On the other hand, the central portion of the mass was composed of sheet-like and/or nest-like structures, containing large atypical polygonal cells, with relatively loose connections among the tumor cells (Figure 2C). They infrequently displayed rosette-like structures, but the central necrosis was frequently seen. Immunostaining indicated that they were positive for NCAM and CK8, and partially positive for synaptophysin and INSM1 (Figure 2D). Only a few cells were positive for chromogranin-A. On the other hand, the tumor cells were entirely negative for CK5/6, p63, and p40, which are squamous cell markers. Therefore, this component was considered to be an LCNEC.

The Ki-67 labeling indices of the SqCC and LCNEC were 51% and 68%, respectively. The border between the two components was irregular, but the components could be clearly divided via immunostaining (Figure 2E). No obviously transitional zone was observed. As the SqCC component was strongly positive for p53 and the LCNEC component was weakly positive for p53, the SqCC displayed a mutation pattern, and the LCNEC exhibited the wild type pattern (Figure 2F and 2G).

We finally diagnosed the patient with HC composed of SqCC and LCNEC.

Case 3

Clinical findings

The patient was a 66-year-old Japanese male, who had developed a swollen mass on the right side of his neck. As the mass enlarged rapidly and was painful when pressure was applied to it, the patient was admitted to a local clinic, and a FNAB resulted in a diagnosis of a “malignant tumor, suspected, especially, SDC”. The patient was admitted to our hospital one month later. An immovable mass was present in the right parotid region, and the patient was experiencing pain and facial nerve paralysis. Under a clinical diagnosis of right parotid gland cancer (pT4aN2bM0), total parotidectomy with skin and facial nerve resection was performed. Postoperative chemoradiotherapy was administered, but the patient stopped attending follow-up examinations after 2 years.

Pathological findings

Macroscopically, an ill-defined yellowish-white mass, which exhibited partial lobulated growth and slight cystic changes, was seen (Figure 3A). The tumor measured approximately 3.3 x 3.0 x 4.8 cm.

Histologically, the tumor was composed of a combination of an SDC and an EMC. The EMC component, which accounted for 55% of the tumor, exhibited the typical histology; i.e., it demonstrated a bi-phasic growth pattern, involving inner ductal cells and outer clear cells (Figure 3B). These cells displayed mild to moderate cellular atypia, and a low mitotic rate, and apocrine differentiation of the inner ductal cells, e.g., eosinophilic cytoplasm and sprouting on the luminal side, was also observed. Immunostaining showed that the inner cells of this component were positive for EMA and CK7 (Figure 3C) and partially positive for CK5/6, whereas the outer clear cells were positive for CK14, CK5/6, alpha-smooth muscle actin (α-SMA), p63, p40, vimentin, and Wilms’ tumor (WT)-1 (Figure 3D). The inner cells were also positive for EGFR, but they were negative for HER2. Therefore, this component was considered to be an EMC.

Approximately 45% of the tumor was composed of proliferating atypical ductal cells, which exhibited marked cellular atypia and eosinophilic cytoplasm. The tumor cells displayed the cystic-papillary, Roman bridge, and solid nest patterns, as well as partial comedonecrosis (Figure 3E). The mitotic rate was relatively high. Immunostaining indicated that they were positive for EMA, CK7, AR, GCDFP-15, and HER2 (2+) (Figures 3F and G), but negative for EGFR and myoepithelial markers. Therefore, this component was considered to be an SDC.

A transitional zone between the SDC and EMC was observed. Atypical apocrine cells were present among the clear myoepithelial cells of the EMC component (Figure 4). As both components were focally positive for p53, they were considered to display the wild-

type pattern. The Ki-67 labeling indices of the SDC and EMC components were 23% and 18%, respectively (Supplemental figure 12A and 1B). Therefore, according to Takase's classification [26], the SDC component belonged to apocrine A subtype.

We finally diagnosed the patient with HC composed of EMC and SDC.

Discussion And Conclusions

Salivary gland HC is an extremely rare and unique neoplasm, and, to the best of our knowledge, only 38 cases have been reported in the English literature, as shown in Table 1[1, 3–23]. Various combinations of carcinoma have been described in HC, and SDC, EMC, and adenoid cystic carcinoma (AdCC) are frequently involved. However, as the tubular subtype of AdCC often exhibits EMC-like features, such as a bi-phasic structure, involving inner ductal cells and clear outer cells[27], HC should be diagnosed carefully in cases involving a combination of EMC and AdCC.[28] HC should not be confused with collision tumors, which arise from two different adjacent tumors. Kufeld et al. described a collision tumor consisting of an AdCC of the pharynx and an SqCC of the larynx [29]. In addition, HC should be distinguished from carcinomas with bi-phasic differentiation, which are single entities composed of two different cellular types, such as EMC. Furthermore, carcinoma with high-grade transformation (HGT) also needs to be differentiated from HC [30]. Several salivary gland tumor entities that exhibit two or more different morphologies should also be ruled out before a diagnosis of HC is made. These entities include collision tumors, synchronous tumors, multiple tumors[31], carcinoma with metaplastic changes, carcinoma with HGT (dedifferentiated carcinoma)[32], and the sarcomatoid variant of SDC [33]. As all of our cases involved two distinct carcinomas in the same topological area, which had combined to produce a single tumor mass, they were defined as HC.

The presence of a transitional zone between two carcinoma components (like in case 3) suggests that they both have the same origin; therefore, detecting such regions allowed us to differentiate our cases from collision tumors, which involve two carcinomas arising at independent topological sites and then subsequently coming into contact. It also allowed us to distinguish them from synchronous and multiple tumors. On the other hand, SDC, SqCC, and LCNEC are entirely of epithelial origin, whereas EMC is composed of both epithelial and myoepithelial cells. Therefore, the presence of a combination of SDC and SqCC (like in case 1) suggests that both components might not be of identical origin, but rather that the SqCC might have arisen through squamous metaplasia in SDC. However, we could not find any histological or immunohistochemical evidence to support this. On the other hand, cases 2 and 3 consisted of a combination of SqCC and LCNEC, and SDC and EMC, respectively. In case 2, an intermingled area was seen between two carcinoma components, and one component might have arisen from the other. In case 3, atypical inner cells with apocrine features were frequently seen in the EMC component; thus, the SDC might have arisen from the EMC.

Nagao et al. proposed that if each component occupied >30% of the tumor mass and could be separated from the other component and the proliferative activity of the two components was markedly different, HC should be considered [3]. In case 1, the SqCC component only accounted for 20% of the tumor mass. However, as the SqCC showed a typical histology and the keratinization observed in the SqCC is not seen in squamous metaplasia of SDC, we considered that this case should be diagnosed as HC. Furthermore, in HC both components seem to display similar levels of proliferative activity. If one component of a suspected HC has high proliferative activity and the other has low proliferative activity, carcinoma with HGT should also be considered [32] As both components exhibited similar levels of proliferative activity in all of our cases, we considered that they were not carcinomas with HGT. Recently, Hamamoto et al. reported a rare case in which SDC arose from EMC, and they considered that their case should be diagnosed as HC rather than carcinoma with HGT, despite the fact that the two components displayed different proliferative activities and histological grades[23]. Our case 3 might have undergone a similar tumorigenic process to their case.

Our case 2 involved a combination of SqCC and LCNEC, and no other cases involving such a combination have been reported. In the current version of the WHO tumor classification, LCNEC was included as a type of poorly differentiated carcinoma [34]. However, we consider that in cases that are positive for neuroendocrine markers, "neuroendocrine carcinoma, including LCNEC," rather than merely "poorly differentiated carcinoma" should be used as a pathological diagnostic term. Salivary gland LCNEC itself is extremely rare. To the best of our knowledge, only 8 cases of LCNEC have been reported in the English literature [35–38]. Salivary gland LCNEC usually exhibits a worse prognosis than other salivary gland carcinomas and a predilection for males. On the other hand, SqCC of the salivary glands is rare, and it is important to always exclude metastatic or invasive SqCC from other

sites [39], such as the oral cavity, oropharyngeal region, head and neck, or skin like Merkel cell carcinoma. In case 2, there was no evidence of other primary sites on PET or other systemic examinations, and the immuno-negativity for CK20 indicated that this tumor was not Merkel cell carcinoma of the skin, and as the tumor was composed of a combination of SqCC and LCNEC, the parotid gland was considered to be the primary site in this case. This is the first reported case of HC involving a combination of SqCC and LCNEC.

Although the previously reported cases of HC frequently showed poor outcomes [3–23], the outcomes of our cases were better; i.e., no recurrence or distant metastasis was seen. Although little information is available, several investigators have suggested that the aggressiveness of HC is determined by the histologically higher-grade component.[23] It would appear that treatments for HC should be targeted at the higher-grade component, but this requires further confirmation in much larger series with longer follow-up periods.

In conclusion, 3 HC cases, which involved combinations of SDC and SqCC, SqCC and LCNEC, and SDC and EMC, respectively, were reported, and such cases are extremely rare. Although our cases showed better prognoses than previously reported HC cases, long follow-up is needed. HC might originate as one pre-existing carcinoma and then might acquire another differentiation direction early in the carcinogenic process, producing two components in a single mass.

List Of Abbreviations

HC: hybrid carcinoma, SDC: salivary duct carcinoma, SqCC: squamous cell carcinoma, LCNEC: large cell neuroendocrine carcinoma, CT: computed tomography, AdCC: adenoid cystic carcinoma, EMC: epithelial-myoepithelial carcinoma, CT: computed tomography, MRI: magnetic resonance imaging, PET: positron emission tomography, FNAB: fine-needle aspiration biopsy, CK: cytokeratin, GCDFP-15: gross cystic disease fluid protein-15, AR: androgen receptor, EGFR: epidermal growth factor receptor, HER2: human epidermal growth factor receptor 2, NCAM: neural cell adhesion molecule, INSM1, insulinoma-associated molecule 1

Declarations

Ethical approval and consent to participate

This study was approved by the institutional review board of Shizuoka General Center (SGHIRB#2019007) and Yokohama City University (B191000001). All subjects signed informed consent forms to participate.

Consent for publication

Written informed consent for the publication of clinical details and/or clinical images was obtained from the patients. A copy of the consent form is available for review by the Editor of this journal.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests related to this study.

Funding

The authors declare that they received no funding support for this study.

Authors' contributions

KK designed and drafted the manuscript. KK, SY, AkM, AyM, KA and MS made the histopathological diagnoses. MY and SY collected the clinical data. KH and AK performed the excellent immunohistochemistry. MS supervised this manuscript. All of the authors have read and approved the final manuscript.

Acknowledgements

The authors thank Mr. Naofumi Ishikawa, Ms. Yoko Yamazaki, Mr. Tomohiro Iwasaki, Ms. Chinatsu Tsuchiya, Mr. Kensuke Shimazaki, Mr. Koji Takahashi, Ms. Nobuyo Tsujino, and Mr. Yohei Saguchi, the medical technologists at the Department of Pathology, Shizuoka General Hospital, Shizuoka, Japan, for their excellent technical assistance, and we are grateful to Ms. Mamiko Uemura, the secretary at the Department of Pathology, Shizuoka General Hospital, for preparing the manuscript.

References

1. Seifert G, Donath K. Hybrid tumours of salivary glands. definition and classification of five rare cases. Eur J Cancer B Oral Oncol. 1996; 32: 251-9.
2. Zhou Y, Duarte EM, Eleff DJ, Tafe LJ, Leibowitz JM, Kerr DA. An unusual hybrid salivary gland tumor: Molecular analysis informs the potential pathogenesis of this rare neoplasm. Case Reports in Pathol 2019; http://DOI.org/10.1155/2019/2713234.
3. Nagao T, Sugano I, Ishida Y, Asoh A, Munakata S, Yamazaki K, et al. Hybrid carcinomas of the salivary glnads: report of nine cases with a clinicopathologic, immunohistochemical,a nd p53 gene alteration analysis. Mod Pathol 2002; 15: 724-33.
4. Chetty R, Medley P, Essa A. Hybrid carcinomas of salivary glands. Arch Pathol Lab Med 2000; 124: 494-6.
5. Croitoru CM, Suarez PA, Luna MA. Hybrid carcinomas of salivary glands. Report of 4 cases and review of the literature. Arch Pathol Lab Med 1999; 123: 698-702.
6. Murphy JG, Lonsdale R, Premachandra D, Hellquist HB. Salivary hybrid tumor: adenoid cystic carcinoma and basal cell adenocarcinoma. Virchows Arch 2006; 448: 236-8.
7. Piana S, Damiani S, Asioli S, Magrini E, Barbieri W, Cavazza A. Epstein-Barr-positive lymphoepithelial carcinoma and epi-myoepithelial carcinoma of the parotid gland; a hitherto unreported example of hybrid tumour. Virchows Arch 2004; 445: 425-8.
8. Ruiz-Godoy LM, Mosqueda-Taylor A, Suarez-Roa L, Poitevin A, Bandala-Sanchez E, Meneses-Gacrcia A. Hybrid tumours of the salivary glands. A report of two cases involving the palate and a review of the literature. Eur Arch Otorhinolaryngol 2003; 260: 312-5.
9. Snyder ML, Paulino AF. Hybrid carcinoma of the salivary gland: salivary duct adenocarcinoma adenoid cystic carcinoma. Histopathology 1999; 35: 380-383.
10. Woo JS, Kwan SY, Jung KY, Kim I. A hybrid carcinoma of epithelial-myoepithelial carcinoma and adenoid cystic carcinoma in maxillary sinus. J Korean Med Sci 2004; 19: 462-5.
11. Zardawi IM. Hybrid carcinoma of the salivary gland. Histopathology 2000; 37: 283-4.
12. Kamio N, Tanaka Y, Mukai M, Ikeda E, Kuramochi S, Fujii M, Hosoda Y. A hybrid carcinoma: adenoid cystic carcinoma and salivary duct carcinoma of the salivary gland. An immunohistochemical study. Virchows Arch 1997; 430: 495-500.
13. Ballestin C, Lopez-Carreira M, Lopez JI. Combined acinic cell mucoepidermoid carcinoma of the parotid gland. Report of a case with immunohistochemical study. APMIS 1996; 104: 99-102.
14. Delgado R, Klimstra D, Alborea-Saavedra J. Low grade salivary duct carcinoma. A distinctive variant with a low grade histology and a predominant intraductal growth pattern. Cancer 1996; 78: 958-67.
15. Ellis GL, Auclair P, Gnepp DR eds. Surgical pathology of the salivary glands. Philadelphia, PA, Saunders; 1991
16. Kainuma K, Oshima A, Suzuki H, Fukishima M, Shimojo H, Usami S. Hybrid carcinoma of the parotid gland: report of a case (epithelial-myoepithelial carcinoma and salivary duct carcinoma) and review of the literature. Acta Otolaryngol 2010; 130: 185-9.
17. Mosqueda-Taylor A, Cano-Valdez AM, Ruiz-Gonzalez JD, Ortega-Gutirrez C, Luna-Ortiz K. Hybrid salivary gland tumor of the upper lip or just an adenoid cystic carcinoma? Case report. Med Oral Pathol Oral Cir Bucal 2010; 15: e43-7.
18. Simpson PR, Rutledge JC, Schaefer SD, Andersom RC. Congenital hybrid basal cell adenoma-adenoid cystic carcinoma of the salivary gland. Pediatr Pathol 1986; 6: 199-208.

19. Eichhorn W, Precht C, Wehrmann M, Hickmann E, Eichhorn N, Zeuch J, et al. First description of a hybrid tumor of the sublingual gland. Anticancer Res 2013; 33: 4567-71.
20. Atay G, Cabbarzade C, Geddikoglu G, Hosal AS. Hybrid carcinoma of the parotid gland: ductal carcinoma and myoepithelial carcinoma. Kulak Burun Bogaz Ohtis Derg 2014; 24; 46-9.
21. Sabri A, Bawab I, Khalifeh I, Alam E. Hybrid tumor of the parotid gland: a case report and review of the literature. Case Rep Otolaryngol 2005; DOI: 10.1155/2015/192453.
22. Zhou Y, Martinez Duarte E, Eleff DJ, Tafe LJ, Leibowitz JM, Kerr DA. An unusual hybrid salivary gland tumor: Molecular analysis informs the potential pathogensis of this rare neoplasm. Case Rep Pathol 2019; DOI: 10.1155/2019/2713234, 2019
23. Hamamoto Y, Harada H, Suzuki M, Fujii T, Nakatsuka SI. Salivary duct carcinoma of the parotid gland originating from an epithelial-myoepithelial carcinoma: Report of a rare case. Head Neck Pathol 2019; 14: 283-9.
24. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American society clinical oncology/college of American pathologists clinical practice guideline focused update. J Clin Oncol 2018; 36: 2105-22.
25. Takase S, Kano S, Tada Y, Kawakita D, Shimura D, Hirai H, et al. Biomarker immunoprofile in salivary duct carcinoma: Clinicopathological and prognostic implications with evaluation of the revised classification. Oncotarget 2017; 8; 59023-35.
26. Grenko RT, Abendroth CS, Davis AT, Levin RJ, Dardick I. Hybrid tumors or salivary gland tumors sharing common differentiation pathways? Reexamining adenoid cystic and epithelial-myoepithelial carcinomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 86: 188-195.
27. Bishop JA, Westra WH. MYB translocation status in salivary gland epithelial-myoepithelial carcinoma. Evaluation of classic, variant, and hybrid forms. Am J Surg Pathol 2018; 42: 319-25.
28. Kufeld M, Junker K, Sudhoff H, Dazert S. Collision tumor of a hypopharyngeal adenoid cystic carcinoma and a laryngeal squamous cell carcinoma. Laryngorhinootologie (in German) 2004; 83: 51-4.
29. Tran TAN, Bridge JA, Deharvengt SJ, Green DC, Linos K. Primary cutaneous adenomyoepithelioma ex spiradeoma with malignant histologic features, epithelial-myoepithelial carcinoma type: A first case report with molecular studies. Int J Surg Pathol 2020; 26: 427-38.
30. Gnepp DR, Schroeder W, Heffner D. Synchronous tumors arising in a single major salivary gland. Cancer 1989; 63: 1219-24.
31. Nagao T. “Dedifferentiation” and high-grade transformation in salivary gland carcinomas. Head Neck Pathol 2013; Suppl 1: S37-S47.
32. Nagao T, Gaffey TA, Serizawa H, Iwaya K, Watanabe A, Yoshida T, et al. Sarcomatoid variant of salivary duct carcinoma: Clinicopathologic and immunohistochemical study of eight cases with review of the literature. Am J Clin Pathol 2004; 122: 222-31.
33. Chiosea S, Gnepp DR, Perez-Ordonez B, et al. “Poorly differentiated carcinoma” eds. EL-Nagger A, Chan JKC, Grandis JR, Takata T. Slootweg PJ. WHO classification of head and neck tumours. Lyon, IARCPress, 2017 pp.180-1
34. Nagao T, Sugano I, Ishida I, Tajima Y, Munakata S, Asoh A, et al. Primary large-cell neuroendocrine carcinoma of the parotid gland: immunohistochemical and molecular analysis of two cases. Mod Pathol 2000; 13: 554-61.
35. Casas P, Bermaaldez R, Patron M, Lopez-Ferrer P, Garcia-Cabezas MA. Large cell neuroendocrine carcinoma of the parotid gland: case report and literature review. Auris Nasus Larynx. 2005; 32: 89-93.
36. Kawaratani H, Tsujimoto T, Yoshikawa M, Kawanami F, Shirai Y, Yoshiji H, Morita K, Fukui H. Large cell neuroendocrine carcinoma presenting with neck swelling in the submandibular gland: a case report. J Med Case Res 2013; 19: 81.
37. Yamamoto N, Minami S, Kidoguchi M, Shindo A, Tokumaru Y, Fujii M. Large cell neuroendocrine carcinoma of the submandibular gland: case report and literature review. Auris Nasus Larynx 2014; 41: 105-8.
38. Chiosea S, Hunt JL, Nagao T, et al. “Squamous cell carcinoma” eds. EL-Nagger A, Chan JKC, Grandis JR, Takata T. Slootweg PJ. WHO classification of head and neck tumours. Lyon; IARCPress 2017 pp.182.

Tables

Table 1
Clinicopathological summary of previously reported salivary hybrid carcinomas

No.	Study	Age	Gender	Site	Histology	Therapy	Outcome
1	Seifert & Donath [1]	70	M	Paro	BCA/CA	S	N.A.
2	Seifert & Donath [1]	62	M	Paro	BCA/AdCC	S	N.A.
3	Seifert & Donath [1]	60	M	Paro	WT/SebLyA	S	N.A.
4	Seifert & Donath [1]	53	M	Paro	Acinic/SDC	S	N.A.
5	Seifert & Donath [1]	66	F	Palate	EMC/AdCC	S	N.A.
6	Ellis et al. [15]	N.A.	N.A.	Paro	EMC/AdCC/ BCAC	N.A.	N.A.
7	Delgado et al. [14]	67	M	Paro	SDC/MC	S + ND	DOD (3y3m)
8	Ballestin et al. [13]	67	F	Paro	Acinic/MEC	S	NED (1y6m)
9	Kamio et al. [12]	51	M	Palate	AdCC/SDC	S + ND	DOAD (1y7m)
10	Simpson et al. [18]	62	F	Paro	EMC/AdCC	N.A.	NED (1y8m)
11	Croitoru et al. [5]	53	M	Paro	AdCC/MEC	S + RT	NED
12	Croitoru et al. [5]	71	M	Paro	AdCC/MEC	S + ND + RT	NED
13	Croitoru et al. [5]	28	M	Paro	EMC/SDC	S + RT	AWD
14	Croitoru et al. [5]	51	M	Paro	AdCC/SDC	S + ND + RT	AWD
15	Snyder & Pauline [9]	36	F	SMG	SDC/AdCC	S + ND + RT	AWD
16	Zardawi [11]	78	F	Paro	PLGA/SDC/AdCC/Acnic	S	N.A.
17	Chetty et al. [4]	58	M	Paro	EMC/MEC	N.A.	N.A.
18	Nagao et al. [3]	74	F	Paro	EMC/BCAC	S + RT	NED (10 m)
19	Nagao et al. [3]	56	M	Paro	EMC/BCAC	S + ND + RT	NED (2y7m)
20	Nagao et al. [3]	73	F	Paro	EMC/SqCC	S	NED (4y)
21	Nagao et al. [3]	40	M	Paro	SDC/AdCC	S + RT	NED (15y)
22	Nagao et al. [3]	81	F	SMG	SDC/AdCC	S + ND + RT	N.A.
23	Nagao et al. [3]	65	M	Paro	MC/SDC	S + ND + RT	AWD (4 m)
24	Nagao et al. [3]	42	M	Paro	Acinic/SDC	S	N.A.
25	Nagao et al. [3]	66	M	Paro	SqCC/SDC	S + ND + RT	AWD (1y8m)
26	Nagao et al. [3]	64	F	LacG	SqCC/SDC	S	NED (7 m)
27	Woo et al. [10]	26	F	Max Sinus	EMC/AdCC	S + RT + Ch	DOD (7y)
28	Ruiz-Goloy et al. [8]	49	F	Palate	MEC/AdCC	S + ND + RT	NED (10 m)
29	Ruiz-Goloy et al. [8]	71	M	Palate	EMC/AdCC	S + RT	NED (4y1m)

No.	Study	Age	Gender	Site	Histology	Therapy	Outcome
30	Piana et al. [7]	N.A.	F	Paro	EMC/LEC	S + ND	NED (6 m)
31	Murphy et al. [6]	68	F	Paro	AdCC/BCAC	S	DOAD (5 m)
32	Kainuma et al. [16]	74	M	Paro	EMC/SDC	S	NED (1y4m)
33	Mosqueda-Taylor et al. [17]	65	M	Upper lip	AdCC/EMC	S	NED (3y4m)
34	Eichhorn et al. [19]	56	F	SLG	AdCC/SDC	S + ND + RT	NED (3y)
35	Atay et al. [20]	71	M	Paro	SDC/MC	N.A.	N.A.
36	Sabri et al. [21]	51	M	Paro	EMC/AdCC/BCAC	S + ND + RT	N.A.
37	Zhou et al. [22]	71	M	Paro	MEC/BCA	S + ND + RT	NED (4 m)
38	Hamamoto et al. [23]	71	M	Paro	SDC/EMC	S + RT + Ch	DOD (1y7m)
39	The present case	86	M	Paro	SDC/SqCC	S + ND	NED (2y)
40	The present case	70	F	Paro	SqCC/LCNEC	S + ND + RT	NED (1 m)
41	The present case	66 M		Paro	SDC/EMC	S + ND + Ch	NED ((2y6m)

N.A.: not available; Paro: parotid gland; SMG: submandibular gland; SLG: sublingual gland;

Max Sinus: maxillary sinus; LacG: lacrimal gland;

BCA: basal cell adenoma; CA: canalicular adenoma; AdCC: adenoid cystic carcinoma;

WT: Warthin tumor; SebLyA: sebaceous lymphadenoma;

Acinic: acinic cell carcinoma; SDC: salivary duct carcinoma;

EMC: epithelial-myoepithelial carcinoma; BCAC: basal cell adenocarcinoma;

MC: myoepithelial carcinoma; MEC: mucoepidermoid carcinoma;

PLGA: polymorphous (low-grade) adenocarcinoma; SqCC: squamous cell carcinoma;

LEC: lymphoepithelial carcinoma; LCNEC: large cell neuroendocrine carcinoma;

S: surgery; ND: neck dissection; RT: radiotherapy; Ch: chemotherapy;

DOD: died of disease; AWD: alive with disease; NED: no evidence of disease;

DOAD: died of another disease

Table 2
The antibodies used in this study and the results obtained with them

					Case 1		Case 2		Case 3		
									SDC	EMC	
Antigen	Clone	Source	System	Antigen retrieval	SDC	SqCC	SqCC	NEC		Inner cells	Outer cells
EMA	NCL-EMA	Leica Biosystems (Nussloch, Germany)	L	ER1 (10 min)	++	F+	N.D.	N.D.	+++	++	-
CK7	OV-TL-12/30	DakoCytomation (Carpinteria, CA)	L	ER2 (20 min)	+++	F+	-	-	+++	+++	-
CK8	CAM5.2	Becton, Dickinson and Company (Franklin Lakes, NJ, USA)	L	ER2 (20 min)	+	+	+	++	+++	+++	+
CK5/6	D5/16B4	DakoCytomation (Carpinteria, CA)	L	ER2 (20 min)	-	+++	+++	-	-	-	+++
p63	Dak-p63	DakoCytomation (Carpinteria, CA)	L	ER2 (30 min)	-	+++	+++	-	-	-	+++
p40	ACR3006A	Biocare Medical (Pacheco, CA, USA)	L	ER2 (20 min)	-	+++	+++	-	-	-	+++
CK20	Ks20.8	DakoCytomation (Carpinteria, CA)	L	ER2 (20 min)	N.D.	N.D.	-	-	N.D.	N.D.	N.D.
Vimentin	NCL-L-VIM-V9	Leica Biosystems (Nussloch, Germany)	L	ER1 (20 min)	N.D.	N.D.	N.D.	N.D.	-	-	++
α-SMA	1A4	DakoCytomation (Carpinteria, CA)	L	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	++
CK14	LL002	Leica Biosystems (Nussloch, Germany)	L	ER1 (20 min)	N.D.	N.D.	N.D.	N.D.	-	-	++
WT-1	6F-H2	DakoCytomation (Carpinteria, CA)	L	ER2 (20 min)	N.D.	N.D.	N.D.	N.D.	-	-	++
AR	AR441	DakoCytomation (Carpinteria, CA)	L	ER2 (20 min)	+++	-	N.D.	N.D.	+++	F+	-

SDC: salivary duct carcinoma; SqCC: squamous cell carcinoma; LCNEC: large cell neuroendocrine carcinoma; EMC: epithelial-myoepithelial carcinoma; EMA: epithelial membrane antigen; CK: cytokeratin; α-SMA: alpha-smooth muscle actin; WT-1: Wilms tumor-1; AR: androgen receptor; GCDFP-15: gross cystic disease fluid protein 15; HER2: human epidermal growth factor receptor 2; EGFR: epidermal growth factor receptor; NCAM: neural cell adhesion molecule; SYN: synaptophysin; CGA: chromogranin-A; INSM1: insulinoma-associated molecule 1; L: Leica BOND-MAX automatic immunostainer; D: Dako Autostainer Link48; R: Roche VENTANA BenchMark ULTRA automatic immunostainer; ER1: pH6.0 (Leica); ER2: pH9.0 (Leica); CC1: pH8.5 (Roche); CB: citrate buffer, pH6.0; N.D.: not done; -: negative; W+: weakly positive; F+: focally positive (1–10%); +: partially positive (11–30%); ++: positive (31–50%); +++: diffusely positive (> 51%); WT: wild-type pattern; Mut: mutation pattern

The HER2 scores were determined according to the criteria developed by Wolff et al. [24]

The p53 expression pattern was estimated according to the criteria developed by Boyle et al. [25]

					Case 1		Case 2		Case 3		
GCDFP-15	23A3	Leica Biosystems (Nussloch, Germany)	L	ER1 (20 mm)	+++	-	N.D.	N.D.	+++	F+	-
HER2	(P)	Roche Tissue Diagnostics, Inc. (Indianapolis, IN USA)	R	CC1 (64 min)	0	0	N.D.	N.D.	2+	0	-
EGFR	EGFR.113	Leica Biosystems (Nussloch, Germany)	L	ER2 (20 min)	+	++	N.D.	N.D.	-	-	-
NCAM	1B6	Leica Biosystems (Nussloch, Germany)	L	ER2 (20 min)	N.D.	N.D.	-	++	N.D.	N.D.	N.D.
SYN	SY38	DakoCytomation (Carpinteria, CA)	L	ER2 (20 min)	N.D.	N.D.	-	+	N.D.	N.D.	N.D.
CGA	(P)	DakoCytomation (Carpinteria, CA)	L	ER1 (20 min)	N.D.	N.D.	-	F+	N.D.	N.D.	N.D.
INSM1	A-8	Santa Cruz Biotechnology (Santa Cruz, CA, USA)	L	ER1 (10 min)	N.D.	N.D.	-	+	N.D.	N.D.	N.D.
p53	DO-7	DakoCytomation (Carpinteria, CA)	D	CB (10 min)	F+ (WT)	F+ (WT)	++ (Mut)	F+ (WT)	W+ (WT)	F+ (WT)	
Ki-67	MIB-1	DakoCytomation (Carpinteria, CA)	R	CC1 (64 min)	58	43%	51%	68%	23%	18%	

SDC: salivary duct carcinoma; SqCC: squamous cell carcinoma; LCNEC: large cell neuroendocrine carcinoma; EMC: epithelial-myoepithelial carcinoma; EMA: epithelial membrane antigen; CK: cytokeratin; α-SMA: alpha-smooth muscle actin; WT-1: Wilms tumor-1; AR: androgen receptor; GCDFP-15: gross cystic disease fluid protein 15; HER2: human epidermal growth factor receptor 2; EGFR: epidermal growth factor receptor; NCAM: neural cell adhesion molecule; SYN: synaptophysin; CGA: chromogranin-A; INSM1: insulinoma-associated molecule 1; L: Leica BOND-MAX automatic immunostainer; D: Dako Autostainer Link48; R: Roche VENTANA BenchMark ULTRA automatic immunostainer; ER1: pH6.0 (Leica); ER2: pH9.0 (Leica); CC1: pH8.5 (Roche); CB: citrate buffer, pH6.0; N.D.: not done; -: negative; W+: weakly positive; F+: focally positive (1–10%); +: partially positive (11–30%); ++: positive (31–50%); +++: diffusely positive (> 51%); WT: wild-type pattern; Mut: mutation pattern

The HER2 scores were determined according to the criteria developed by Wolff et al. [24]

The p53 expression pattern was estimated according to the criteria developed by Boyle et al. [25]

Figures

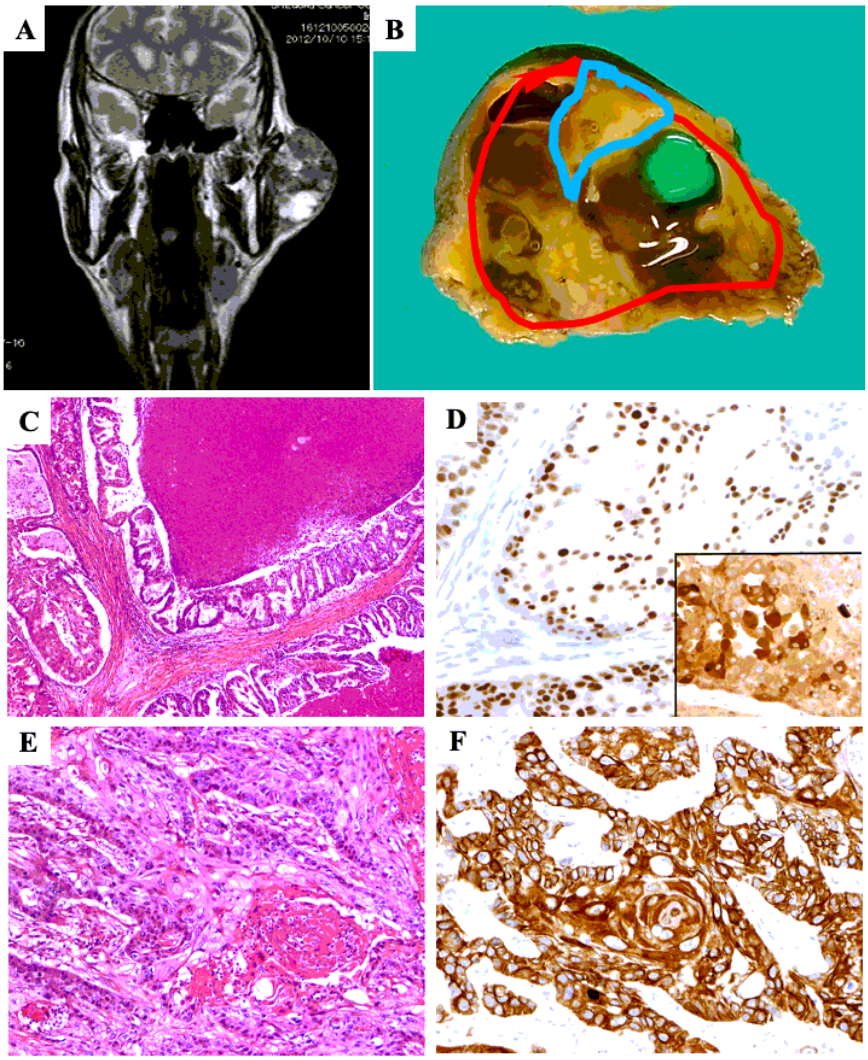

Figure 1

(case 1). (A) On MRI, T2-weighted imaging showed an area of low signal intensity with a focal area of high signal intensity in the left parotid region. (B) Macroscopic mapping: The SDC component accounted for approximately 80% of the tumor (red area), whereas the SqCC component accounted for the remaining 20% (blue area). (C)The SDC cells displayed Roman bridge structures and comedonecrosis (H & E). (D) They also were immunopositive for both AR (Inset: The SDC cells were also positive for GCDFP-15). (E) The SqCC cells demonstrated moderate keratinization and included stratified atypical squamous cells (H & E). (F) They were immunopositive for CK5/6.

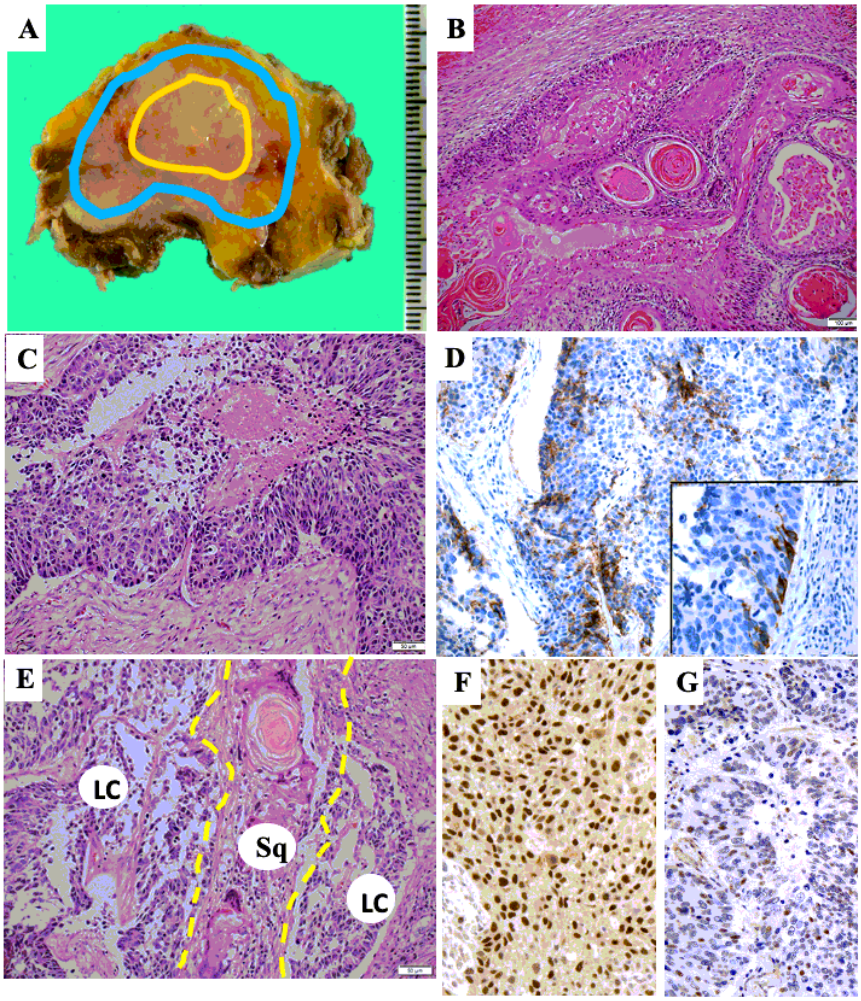

Figure 2

(case 2). (A) Macroscopically, a relatively ill-defined mass was seen in the left parotid gland. The blue area indicates the SqCC component, whereas the yellow area represents the LNCEC component. (B) The SqCC cells displayed marked keratinization and included stratified atypical squamous cells (H & E). (C) On the other hand, the LCNEC cells exhibited sheet-like or nest-like growth, involving less-cohesive atypical cells with central necrosis (H & E). (D) The LCNEC cells were immunopositive for NCAM (Inset: The cancer cells were also focally positive for synaptophysin). (E) Histologically, the border area between the SqCC (Sq) and LCNEC (L) components was irregular (H & E). The SqCC cells were strongly positive for p53 (F), whereas the LCNEC cells were weakly positive for p53 (G).

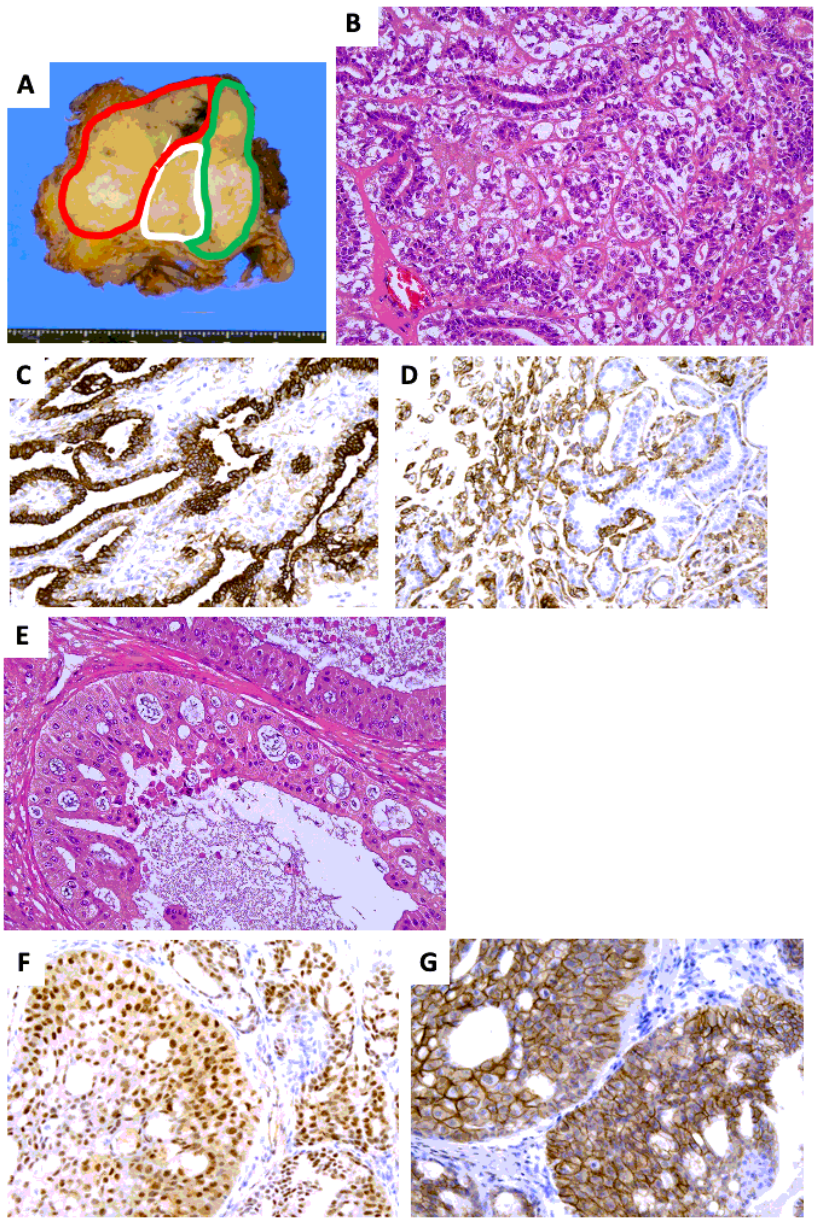

Figure 3

(case 3). (A) Macroscopically, one lobulated mass was seen. However, the red area was an SDC component, whereas the green area was an EMC component. The white area represents the transition zone between the SDC and EMC components. (B) The EMC component had a two-phase structure, composed of inner small luminal cells and outer clear large cells (H & E). (C) The inner cells were immunopositive for CK7. (D) The outer cells were immunopositive for α-SMA. (E) The SDC component consisted of Roman bridge structures and comedonecrosis, involving large atypical eosinophilic cells (H & E). The cancer cells were immunopositive for AR (F) and HER2 (G).

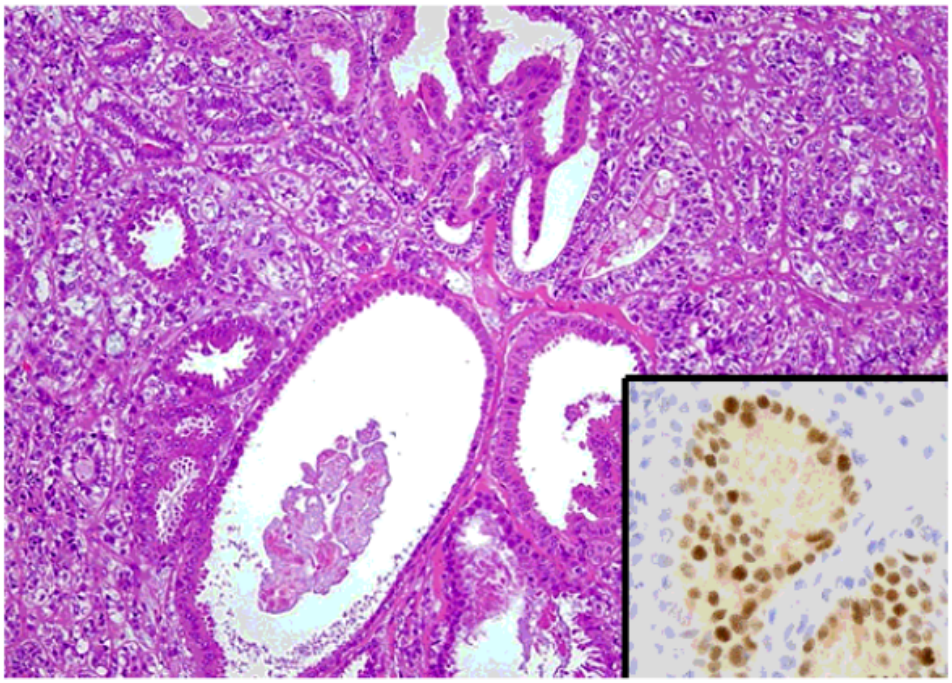

Figure 4

(case 3). On the other hand, the transition zone was composed of a two-phase structure, involving the relatively large atypical eosinophilic luminal cells with sprouting and the clear outer cells with moderately cellular atypia (H & E: Inset: the inner atypical cells were positive for AR).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CAREchecklistEnglish2013.pdf
- SuppliFig1.tiff
- Supplementalfigureslegend.docx