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Case Report Open Access

Combined Small Cell Carcinoma and Squamous Cell Carcinoma of the Parotid Gland: Immunohistochemical and Molecular Characterization

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Abstract

Combined SCC and SqCC of salivary gland are defined as a composite tumor and it represents an even more rare entity, often recognized just after evaluation of the surgical specimen. Here we report an extraordinary case of combined SCC and SqCC of the parotid gland in a 73-year-old man with a prior medical history of cutaneous squamous cell carcinoma. A computed tomography scan showed a 3 cm-sized heterogeneous mass in the parotid region while no other primary tumor or metastasis and lymphadenopathy were detected. The patient underwent to a partial parotidectomy and morphological and immunohistochemical features of the tumor were consisting in combined SCC and SqCC. SqCC component was immunoreactive for p40 whereas SCC neoplastic cells resulted strongly positive either for synaptophysin and chromogranin and negative either for p40 or high molecular weight cytokeratins. To identify the main putative genetic alterations specific for each histotype component, we performed a molecular profiling of *FGFR1, MET, MYC, TP53, BRAF, NOTCH1, EGFR, NFE2L2, PDGFRa, cKIT*, and *PIK3CA* genes. Interestingly, these analyses did not disclose any known genetic alterations in any neoplastic component of the combined tumor. To the best of our knowledge, this is the first detailed report of a combined small cell and squamous cell carcinoma of the parotid gland. Our case did not show any known molecular signature. Further investigation will clarify the pathogenesis and relationship of the individual components and will be of great utility for the treatment of these extremely rare entity..

Keywords: SCC; SqCC; Parotid gland

Introduction

Currently, studies assessing combined small-cell carcinoma (c-SCC) are limited, therefore its clinic-pathological features, treatment, and prognosis have not been fully determined. SCC is known as a malignant epithelial tumor that shows neuroendocrine differentiation. It originates mainly in the lung and its occurrence in head and neck is a rare clinical entity, histologically similar to small cell lung carcinoma [1]. SCC of the salivary gland is an extremely rare and aggressive tumor, accounting for less than 1% of all salivary gland tumors and fewer than 2% of salivary gland malignancies [2]. Due to the extreme rarity of these cases, no definite strategic treatment regimen has been well defined. Primary squamous cell carcinoma (SqCC) of major salivary glands is rare and accounts for about 1.6% of salivary gland neoplasms, whereas neuroendocrine tumors, SCC and combined-type SqCC and atypical carcinoids in the head and neck area were reported only in few cases [3-7].

Here we report an extraordinary case of combined SCC with SqCC of the parotid gland, with special reference to the pathological, immunohistochemical and molecular characterization.

Case Presentation

A 73-year-old male was referred to the Head and Neck Department of Fondazione IRCCS Casa Sollievo della Sofferenza complaining of a 3-month history of a rapidly enlarging painful mass in the right parotid gland. Few months before he underwent to the resection of a cutaneous squamous cell carcinoma of the right ear. A CT scan revealed in the parotid region a heterogeneous solid mass measuring 3 cm in diameter, with lobular contours and nonspecific borders (Figure 1). No regional lymph node enlargement was detected. The chest/abdomen CT was normal, and no other primary tumor or metastasis were found. A fine-needle aspiration biopsy (FNAB) revealed malignant cells. The patient underwent to a partial parotidectomy with preservation of the facial nerve and the surgical specimen consisted of a biphasic tumor represented by SqCC combined to an undifferentiated neoplastic component. The squamous cell carcinoma was immunoreactive for p40 whereas undifferentiated neoplastic cells resulted strongly positive either for synaptophysin and chromogranin and negative either for p40 or high molecular weight cytokeratins (clone 34betE12) consisting with a diagnosis of combined SCC and SqCC (Figure 2).

A differential molecular profiling of the two neoplastic components was performed searching for specific SqCC and SCC genetic aberrations. The presence of *FGFR1* (8p11-23-p11.22), *MET* (7q31.2), and *MYC* (8q24.21) amplifications was assessed in the pathological specimens by Fluorescent *in Situ* Hybridization (FISH) analysis using Vysis LSI FGFR1 SpectrumRed Probe/Vysis CEP 8 SpectrumAcqua, Vysis MET SpectrumRed FISH Probe FISH Kit, and Vysis LSI MYC Spectrum Orange/Vysis CEP8 Spectrum Green Probes Kit (Abbott Molecular, Abbott Park, IL, USA) respectively. FFPE tissue sections (10 µm thick) were microdissected separately to enrich tumor cells and obtain DNA sample for each tissue type. DNA was extracted according

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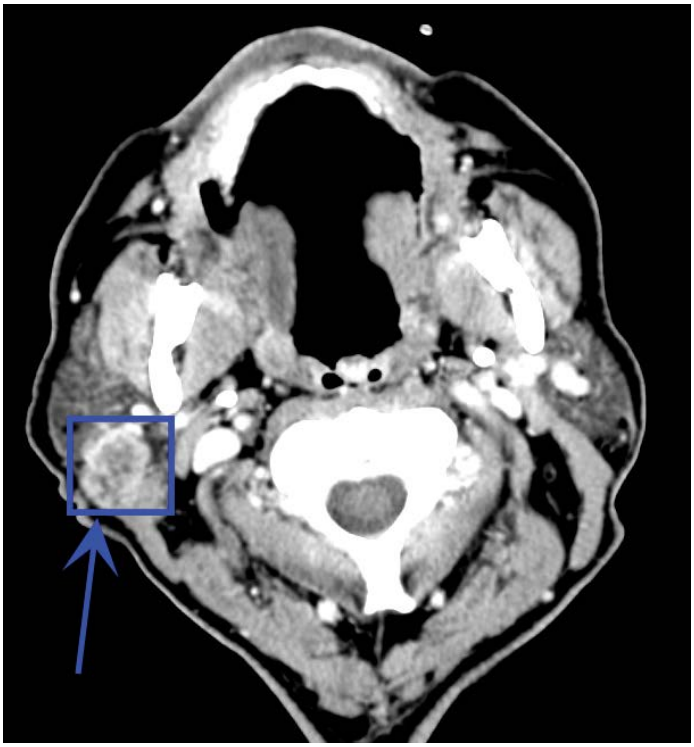

Figure 1: Pre-operative CT scan with contrast (axial plane) showing a 3 cm lesion of the right parotid with irregular margins and a necrotic center (blue arrow and square).

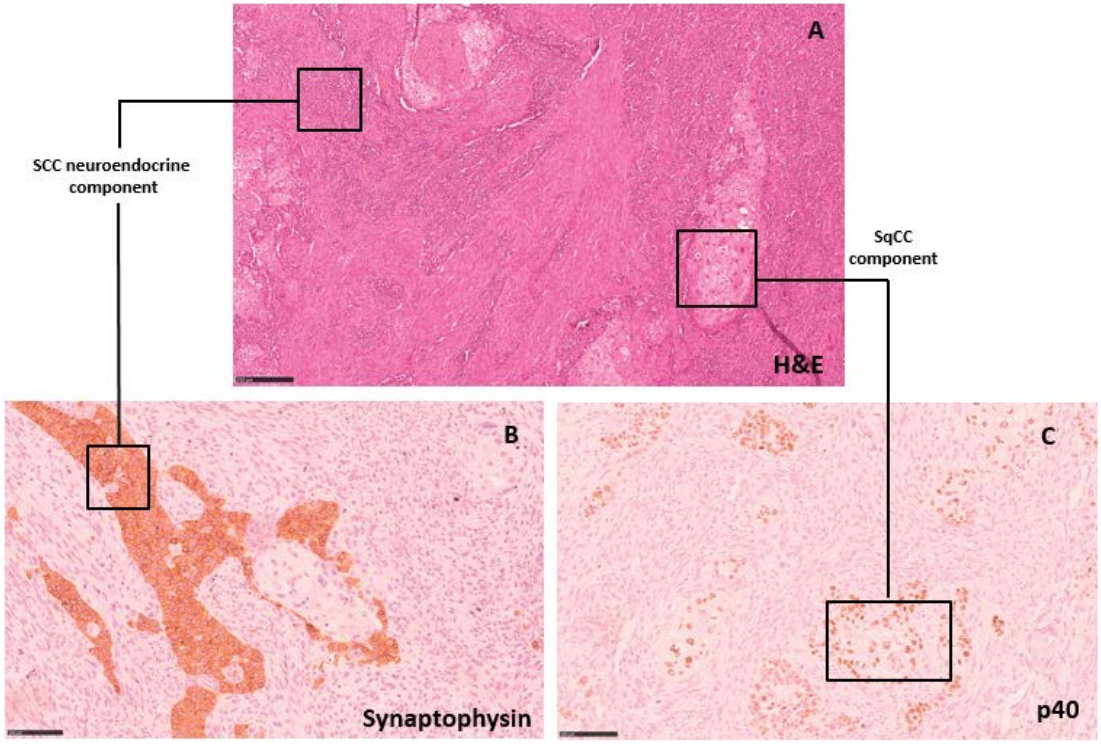

Figure 2: Microscopically, the tumor consisted of (A) a keratinizing squamous cell carcinoma (SqCC) combined to an undifferentiated small cell carcinoma (SCC); (B) SqCC component was immunoreactive for p40 (X40); (C) SCC neoplastic cells resulted strongly positive for synaptophysin.

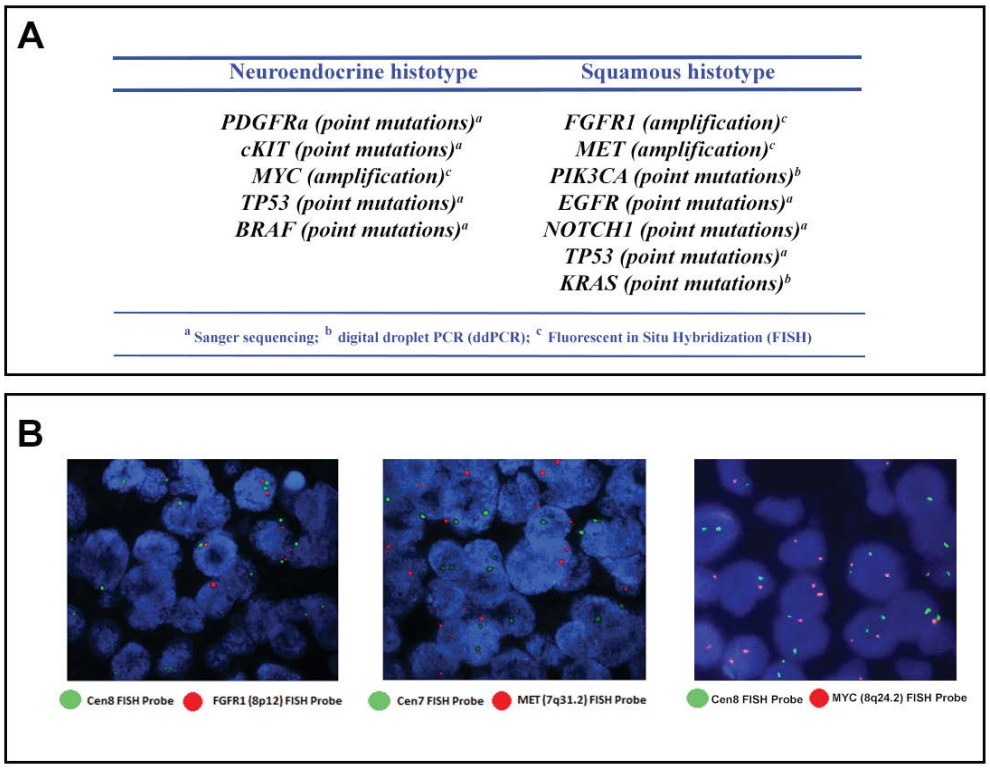

Figure 3: Molecular characterization of the SCC and the SqCC components. (A) List of the genes analyzed for each pathological histotype [experimental methods: a- Digital droplet PCR (ddPCR); b-Fluorescent *in situ* Hybridization (FISH)]. (B) Results of FISH experiments performed to assess the amplification status of *FGFR1* (left), *MET* (middle), and *MYC* (right). The results of the FISH tests show the negativity for *FGFR1*, *MET* and *MYC* amplifications.

to the manufacturer's instructions by using the GeneRead DNA FFPE kit (Qiagen, Valencia, CA, USA) and then quantified on Qubit 3.0 Fluorometer with the Qubit dsDNA BR assay Kit (Life Technologies by Thermo Fisher Inc). PCR amplifications of *TP53* exons 4-8, *BRAF* exon 15, *NOTCH1* exon 26, 27, 34, *EGFR* exons 19-21, and *NFE2L2* exon 2, *PDGFRa* exons 12,14 and 18, and *cKIT* exons 9, 11, 13, 14, 17 were performed for both DNA sample as previously described and the gene regions sequenced by Sanger approach on an ABI 3100 capillary sequencer (Applied Biosystems) and by the Sequencing Analysis software v.3.7 (PE Applied Biosystems) [8-12]. DNA extracted from the two different cancer histotype portions was also analyzed by digital droplet PCR (ddPCR) by using the QX100™ Droplet Digital™ PCR System (Bio-Rad, Hercules, CA, USA) with Primer and probes for *KRAS* and *PIK3CA* ddPCR mutation assays were from Bio-Rad (www.bio-rad.com/it) to detect putative mutation on exon 2 of *KRAS* and on exons 9 and 20 of *PIK3CA* genes. No *FGFR1* or *MET* or *MYC* genes amplifications were detected by FISH (Figure 3). Moreover, no mutations were disclosed in *TP53, BRAF, NOTCH1, EGFR, NFLE2, KRAS* and *PIK3CA* genes by PCR analyses.

Because of the clinical status, the patient was not considered for adjuvant radiotherapy. Three months later a CT scan performed during routinely follow up showed the presence of multiple pathologic lymph nodes in the right neck and a functional neck dissection was performed. During the same surgical procedure, a cutaneous lesion of the paralatero-nasal area was removed. The histopathological examination showed multiple lymphonodal metastasis of combined SCC and SqCC. The cutaneous lesion resulted as SqCC. The patient underwent adjuvant radiotherapy at a dose of 60 Gy in 30 fractions of the right neck. Five months after the end of radiant treatment, the patient was admitted to the hospital with cerebral metastasis (2 cm nodular lesion involving the left temporal area) demonstrated by Magnetic Resonance Imaging (MRI) and the patient was submitted to Hypo-fractionated stereotactic radiotherapy (hFSRT) with a 30 Gy distant treatment failure (DTF). One month later a new CT total body showed the presence of multiple nodular lung lesions. The Fine Needle Aspiration Biopsy (FNAB) revealed SCC localizations. A combination chemotherapy with Carboplatin and Etoposide treatments was started but three months later (13 months after the first diagnosis) the patient died of complications related to metastatic disease.

Discussion

Neuroendocrine tumors represent a rare, heterogeneous subset of the parotid malignancies and are classified into distinct groups. The oral cavity is a rare site of a primary neuroendocrine tumors and few combined cases of SCC and SqCC in the head and neck district/area were documented to date. A case of combined SCC with SqCC was reported by Aggarwal and colleagues in larynx [13]. They found that the tumor was mainly composed of small cell neuroendocrine carcinoma nearly confined to the right side and involving the supraglottis and invasive SqCC component located on the left side of the larynx (mainly in the glottis). Kołodziej and co-workers described a 58-year-old man diagnosed with a larynx tumor with metastatic neck lymph nodes on the left side without distant metastases [14]. The patient underwent to a total laryngectomy with lymphadenectomy. The postoperative

histologic examination disclosed a combined small cell carcinoma and squamous cell carcinoma. Both tumor components showed clear boundaries; however, foci of gradual transition from one to the other tumor were also detected. The immunohistochemical staining panel showed a diffuse positive reaction to synaptophysin and chromogranin A in the small cell neuroendocrine type carcinoma component. The cells from this component also showed positive reaction to p16, bcl-2 and thyroid transcription factor 1 (TTF-1), whereas the staining for p63 and high-molecular-weight cytokeratin (HMWCK) was negative. The cells from the SCC component showed positive reaction to p63, to HMWCK and to cytokeratin 5/6 (CK5/6), while TTF-1 and p16 were negative. Chromogranin together with synaptophysin allows confirmation of neuroendocrine differentiation in tumors. Hamamoto and co-workers more recently described a record of nine case of carcinoma with neuroendocrine features encountered during their clinical practice [15]. The cases were diagnosed in accordance with the 2005 WHO classification criteria using primary surgically resected specimens and then re-evaluated by experienced pathologists in accordance with the 2010 WHO classification criteria. Among these, two cases were diagnoses as combined SCC and SqCC, with different clinical course of disease [15,16].

We described an extraordinary case of combined SCC with SqCC of the parotid gland. In order to identify differences in the molecular signature involved into the pathogenesis of this combined tumor, we searched both in SCC and SqCC for some of the well-known genetic alterations implicated in the pathogenesis of each histologic component [17,18]. Specifically, we performed a molecular profiling of *FGFR1, MET, MYC, TP53, BRAF, NOTCH1, EGFR, NFE2L2, PDGFRa, cKIT*, and *PIK3CA* genes. Unfortunately, the case described did not show any mutations in these genes specifically associated to SCC or SqCC that should help to explain the pathogenesis of these two histological components. Since published molecular profile of combined SCC and SqCC cases are few documented, many hypotheses were made on their biological origin. SqCC and SCC could arise from pluripotent cells that differentiated along two distinct paths or the SqCC could have differentiated secondarily from cells arising in SCC. An alternative hypothesis is that SCC derived from pluripotential indifferent cells of either the squamous epithelium. In the present case no transitional part between SCC and SqCC was observed, suggesting the hypothesis of a pluripotent cell in origin.

It is well known that SCC arising at extra-pulmonary sites leads to a poor prognosis for patients and recommendations for the treatment of SCC arising in the head and neck are based primarily on retrospective data from various small case series. Owing to the infrequency of primary SCC of the head and neck, it is very unlikely that any large, controlled study will ever be performed, so more cases of this tumor need to accumulate to clarify biological behaviour and prognosis [15,19,20].

Conclusion

Combined SCC and SqCC of the head and neck is so rare that its treatment is not well established. Retrospective data from various case reports and small case series indicate surgery as the main option. However regional treatment alone may be insufficient. Most patients, including the one reported in the present paper, showed nodal or distant metastases at the early stage after initial surgery and died of the disease within one year from the treatment. Uwa et al. stated that all the reported metastasis deriving from combined SCC and SqCC of the larynx and hypopharynx included SCC components, combined with SqCC only in few cases. Therefore, SCC should be targeted in therapy, and multimodality treatment including surgery, radiotherapy and chemotherapy should be the mainstay in the treatment of this type of tumor.

To the best of our knowledge, the case we presented is the first detailed reported of a combined small cell and squamous cell carcinoma of the parotid gland. Additional molecular investigations (by analyzing alterations in a more consistent number of genes by next generation sequencing) could be helpful to clarify the pathogenesis and relationship of the two histo-pathological type and could be of great utility for the treatment of these extremely rare entity.

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Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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