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Case Report

Metastatic salivary ductal carcinoma androgen receptor-positive with V600E BRAF gene mutation

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Abstract: Over the years, several molecular alterations associated with the pathogenesis and progression of the salivary glands tumors have been well-characterized. Particularly, the mutational status and/or aberrant expression of certain markers, such as EGFR, HER2, cKIT, BRAF and AR, mainly identified in some subgroups of salivary duct carcinomas (SDCs) highly aggressive, currently represent molecular targets for new and efficacious drugs routinely employed in the treatment of some common cancers. We describe the case of 52 years old woman with a palpable mass in the left parotid, with cytological examination suggestive for epithelial neoplasia likely metastasis by papillary thyroid tumor. The final diagnosis was high grade salivary ductal carcinoma with carcinomatous metastasis in 12 lymph nodes and masseter muscle. We decided to verify the presence of specific molecular alterations already described, in in particular HER2, ERbeta, AR protein expression and mutational state of bRAF gene. Immunohistochemical analyses revealed the overexpression of AR (40%), a focal positivity (4%) for HER2 and the overexpression of ERbeta in 80% of the neoplastic cells. Moreover we detected V600E bRaf mutation in our specimen. Several studies report the benefits of anti-androgen therapy in salivary glands tumors histotypes. The patient was enrolled for bicalutamide therapy showing a complete metastatic disease regression after six months. We suggest that the expression analysis of these biomarkers associated with histo-morphological data, could then provide the oncologist the opportunity to create a proper stratification of patients for customized therapies also for salivary gland tumors.

Keywords: Salivary duct carcinomas, molecular alterations, target therapy

Introduction

Ductal carcinomas of the salivary glands (SDC) are rare and highly aggressive tumors with a very poor prognosis. SDC is morphologically similar to high grade Ductal Breast Cancer (DBC) and originates from intra-lobular and interlobular excretory ducts and can present a ductal, papillary, cribriform and solid growth pattern with comedonecrosis [1]. It was firstly described in 1968 [2] and only sporadic cases have been reported in literature until today [3-5]. SDC predominantly occurs in males around the sixth decade of life. The parotid is the most affected organ and at the time of diagnosis the majority of patients are in an advanced stage of disease with frequent involvement of lymph nodes of the neck area, with the presence of distant metastases too [1]. Distant metastases occur in 27% of cases [6] and surgical resection of solitary metastasis and chemotherapy may be considered as palliative care [7].

Moreover, histological similarity to ductal carcinoma of the breast has prompted to investigate hormonal receptor status for the development of anti-hormonal therapies. However estrogen alpha (ERa) and progesteron (PgR) receptors expression is absent in most malignant salivary glands tumors [8], androgen receptor, in 67% of cases, and alternative isoform beta of ER (Erbeta) positive in 73% of cases are described [9]. Moreover, similar to DBC, overexpression and amplification of the HER-2 gene were described in respectively in 53.8% and 38.5%

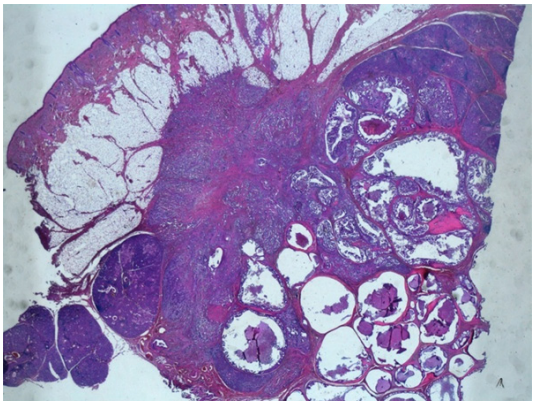

Figure 1. Overview of parotid gland with the infiltrating tumor (2×).

of SDCs [10] and a molecular classification based on immuno-phenotypic pattern was proposed [11].

The recent discovery of specific molecular alterations which involve PIK3CA, PTEN and BRAF V600E kinase genes in a subsets of HER2-negative SDC, has highlighted the molecular complexity of this tumor by opening a new scenario for the use of biological target therapies [12]. Herein we report a case of a patient with a very interesting molecular framework characterised by AR and ERbeta over-expression and V600E mutation of BRAF gene.

Case report

A 52-year-old female presented to our Institute with a metastasis from papillary carcinoma, most likely of thyroid origin, diagnosed on FNAB samples of a latero- cervical lymph node (data not shown).

The patient arrives at our hospital, and after the appropriate diagnostic exams (PET/CT) (data not shown), she underwent to a total excision of the left parotid, latero-cervical and supra-clavear lymph nodes of the neck, facial artery, spinal nerve and masseter muscle.

A total parotidectomy of 7×5×3 cm, with left lymph nodal depletion of 16×10×4 cm with annexed masseter muscle, cm 4×3×2, 5 in size, was grossly analysed. In section, the parenchyma presented a white-grey, seemingly tough nodule, cm 4×2, 5×2 in size. From the fat tissue 15 lymph nodes were isolated and within the muscle tissue two lesions of 2×2 cm with similar characteristics to the main lesion, were also identified.

Histological examination (**Figure 1**) showed a neoplastic proliferation characterized by an intraductal, cribriphorme and solid component with comedonecrosis, and a high grade infiltrative component, consisting of small glands, isolated cells and nests of neoplastic cells with abundant cytoplasm, vesicular nucleus and prominent nucleolus Cellular pleomorphism, high mitotic index (Ki67 = 30%) and intra-tumor necrosis were present; neoplastic intravascular emboli and perineural infiltration were also observed (**Figure 2**). The remaining parenchyma presented chronic sialoadenitis and the pharyngeal extension showed a focus of nodular adenosis.

Immunohistochemical analyses revealed that the neoplastic components, both intraductal one and infiltrating one, showed diffuse and intense positivity for low molecular weight CKs, EMA, GCDFP and AR (40%) (**Figure 3**), while S100, P63, TTF1, ER and PgR were negative. Moreover, HER2 immunostaining showed only a focal incomplete membrane positivity in a rare group of neoplastic cells (4% of the total), while ERbeta expression was detected in 80% of the neoplastic cells (**Figure 4**).

Molecular analyses were also carried out to evaluate V600E mutation of the exon 15 of BRAF gene. The analyses were performed using a commercial kit (BRAF Mutation Analysis Kit, EntroGen, Tarzana, CA, USA), containing a distinct primer/probe in order to detect, by Real Time PCR system, V600 point mutations. The analysis clearly showed the presence of the mutation in our sample (**Figure 5**).

The final diagnosis was high grade salivary ductal carcinoma with carcinomatous metastasis in 12 lymph nodes and masseter muscle. The patient was enrolled for bicalutamide therapy in another Hospital Institution showed a complete metastatic disease regression after six months. Unfortunately, the disease has recently resumed but its evolution is monitored from the hospital where she is currently followed.

Written informed consent was obtained from the patient for publication of this study and the accompanying images and the study was

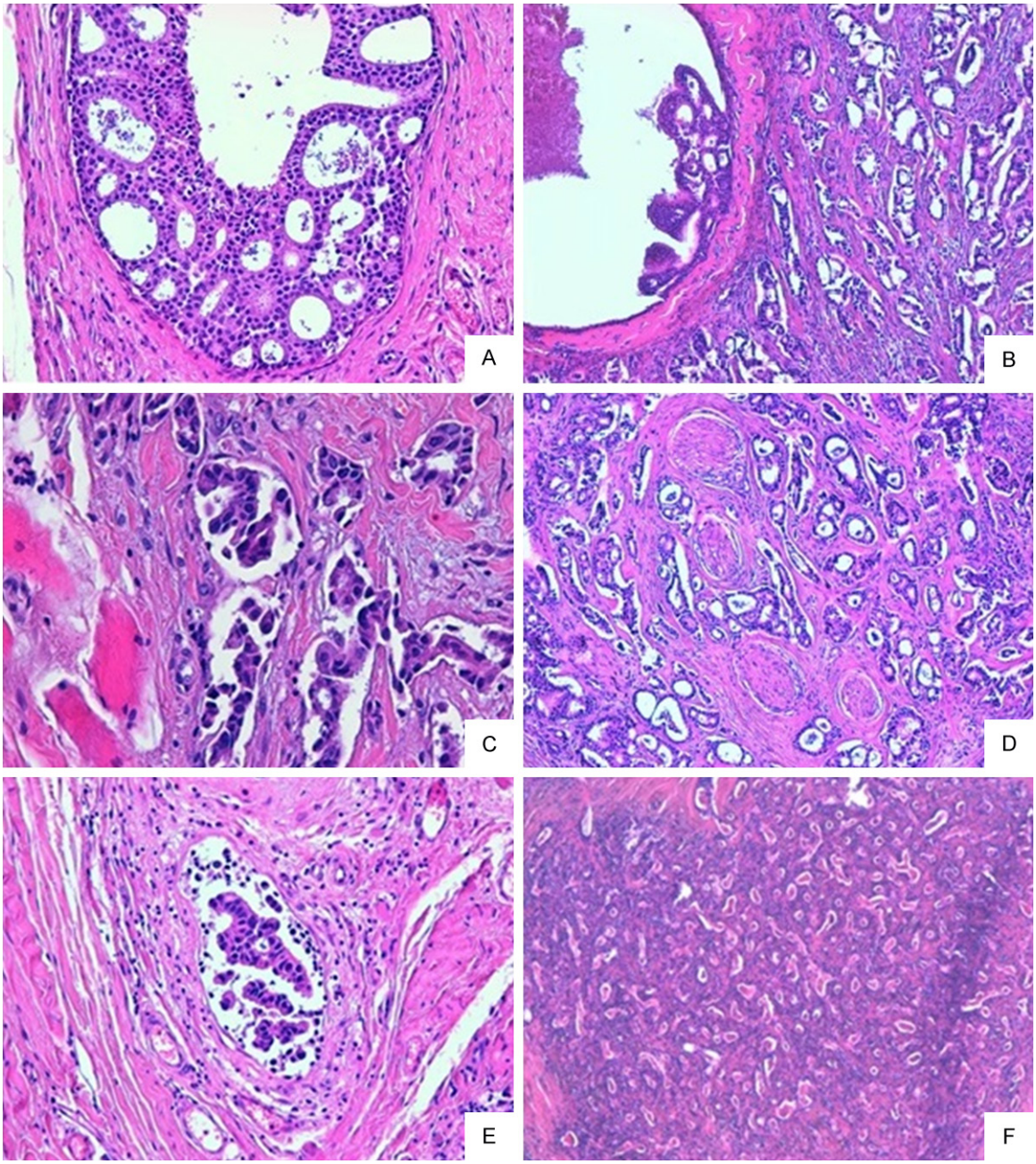

Figure 2. Morphological characteristics of the tumor: A: Detail of cribriphorme pattern (20×); B: Detail of small glands with isolated cells and nest of neoplastic cells; C: Detail of nuclear pleomorphism (20×); D: Detail od penireural infiltration; E: Detail of neoplastic intravascular emboli (20×); F: Detail of nodular adenosis focus (10×).

approved by the ethics committee of the National Cancer Institute "G. Pascale".

Discussion

SDC is one of the most aggressive salivary gland neoplasia, known for high mortality and the presence of distant metastases at the time of diagnosis. It was only recognized as a distinctive clinical-pathologic entity after the revised histologic classification of salivary gland neoplasms by the World Health Organization in 1990 [1].

In the case of a resectable tumor, surgery is the treatment of choice, while primary radiotherapy is recommended in unresectable SDC. Palliative chemotherapy is offered to patients

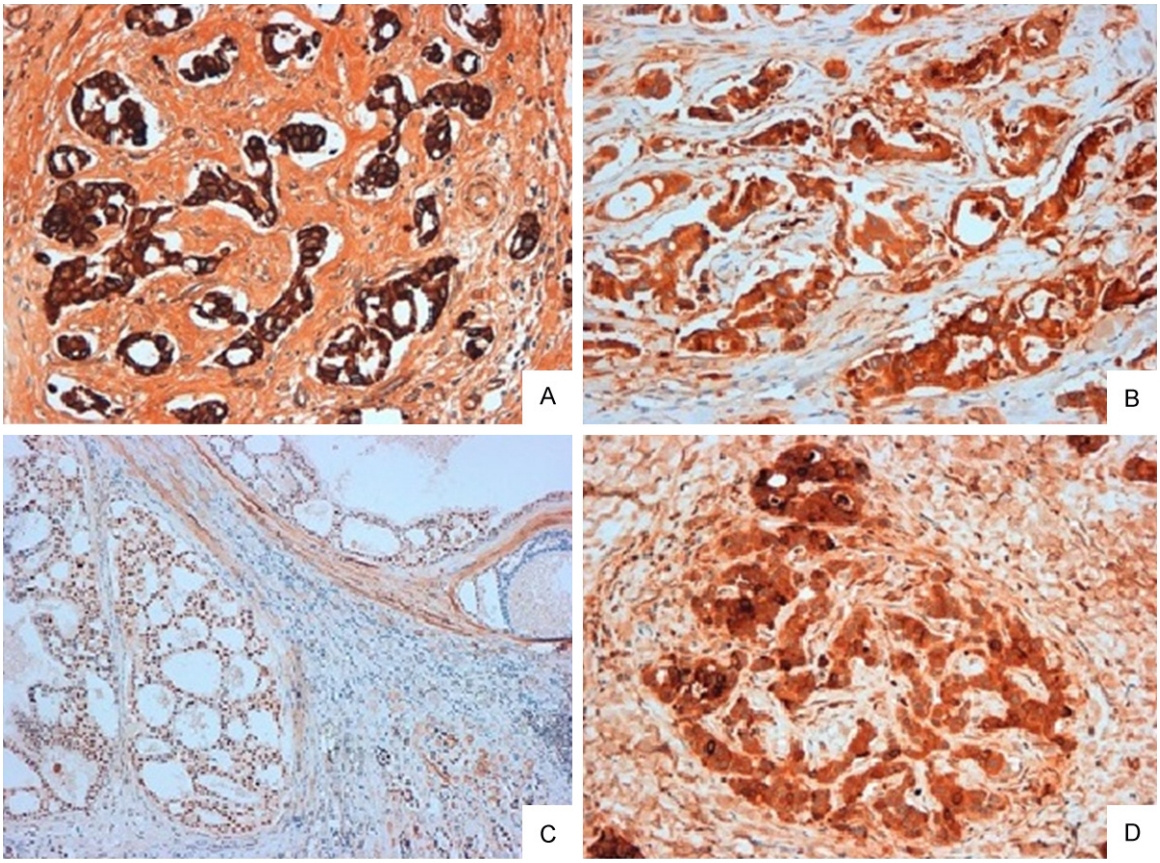

Figure 3. Immunohistochemical features of the lesion: A: Immunopositivity for CK7 (20×); B: Immunopositivity for CEA (20×); C: Immunopositivity for p53 (10×); D: Immunopositivity for GCDFP15 (20×).

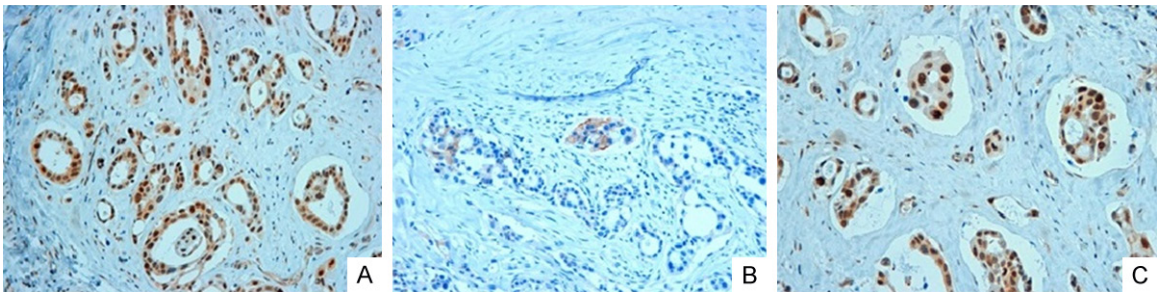

Figure 4. Hormone receptors Immunohistochemical staining: A: Immunopositivity for Erbeta (20×); B: Immunopositivity for HER2 (10×); C: Immunopositivity for AR (20×).

with metastases and unresectable local recurrent disease, but its efficacy remains uncertain [13].

Histologically, SDC resembles high grade Ductal Breast Cancer. As the latter, SDC presents an intra-ductal component, with comedonecrosis too, as well as an invasive component [2]. The neoplastic cells are positive for CK7, GCDFP15, CEA, EMA, AR and negative for ER alfa, PgR, myoepithelial markers (S100-, P63-) and high molecular weight CKs (CK14-CK5/6-) [14]. HER2 over-expression has been described in 53.8% of cases and ERbeta in 73% of SDCs [15]. These markers, in addition to having a therapeutic potential, also set SDCs from a prognostic point of view. In fact, SDCs negative for both AR and ERβ considered more aggressive than tumors which expressed one or both markers. Moreover, SDCs with HER2 protein

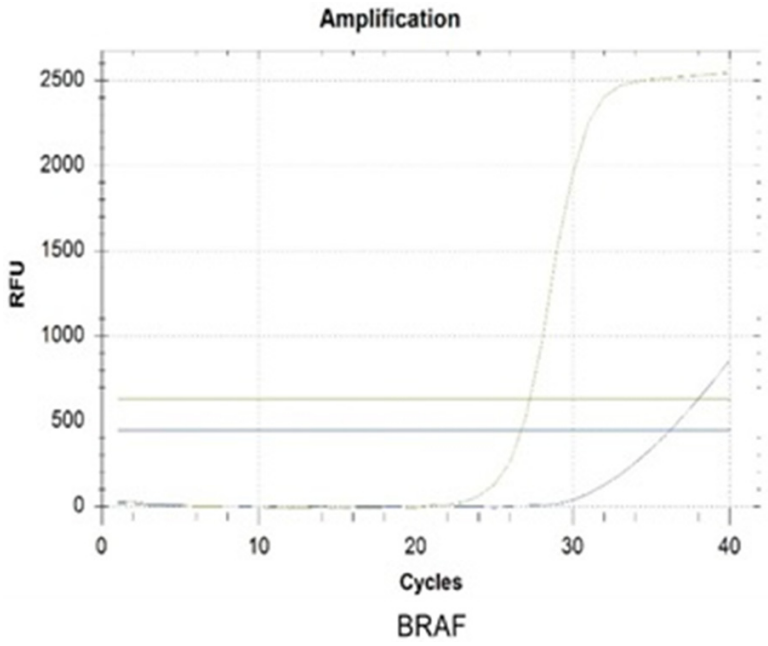

Figure 5. BRAF mutational analysis.

staining 3+ had a poor outcome than those which were HER2 protein 0-2+ [9]. Recently, a molecular classification has been proposed for SDCs, as well as for breast cancers, which divides them, according to the immunohistochemical characteristics, into: luminal androgen receptor positive subtype (AR+, HER2 negative, CK5/6 negative) about 70% of cases; HER2 subtype (HER2/neu gene amplified) 17% of cases; and basal-like phenotype (AR-, HER2-, CK5/6+) 5% of cases [11].

This classification would allow to identify more aggressive molecular histotypes that can take advantage of target therapies, especially in advanced stages of disease. A further support comes from recent studies on genotype analyses that have shown new mutations, such as PIK3CA, PTEN, BRAF V600E in a subset of cases of SDC HER negative [12].

Herein, we have reported a patient with a very complicated clinical history. The initial FNAB analysis led to the assumption of a metastasis from papillary thyroid carcinoma. However, the histo-morphological analysis on surgical tissue samples clearly showed the presence of a SDC. Subsequent analyses have been directed towards the investigations of specific molecular alterations described in literature that would have allowed biological therapies. HER2 evaluation resulted negative, AR was over-expressed in 40% of tumor cells and ERbeta was positive in 80% of tumor cells. Our case, based on new molecular classification, can be framed into Luminal AR subtype, for which the use of anti-androgen drugs has been described as effective and well tolerated therapy [16, 17]. The first documented clinical study reporting the use of androgen-deprivation therapy in a patient with salivary gland carcinoma over-expressing AR, is dated back to 2003 [18].

Subsequently, sporadic case reports and clinic studies have been generated. Jasper and collaborators selected 10 patients with AR-positive SDCs treated with Bicalutamide and described a clinic benefit in 50% of cases [17].

A complete response of a 57-year-old female patient with metastatic SDC, AR positive to Bicalutamide was recently described [19].

Our patient, after histological diagnosis, received Bicalutamide in another Hospital, and showed a complete clinic response after six months. However, her medical history has undergone further changes for the recurrence of the disease, but we cannot document it.

The investigation on specific molecular alterations described for this tumor histotype, as the over-expression of AR, HER2, ERbeta and several gene mutations, could open a new scenario in the therapeutic stratification of patients with SDCs [9]. The use of biological therapies is well tolerated by these patients and very effective. Our patient also revealed the presence of BRAF V600E mutation. Currently, a specific inhibitor of BRAF V600E mutated, vemurafenib [20], was described, used in particular in metastatic melanoma [21]. Nevertheless, a single case of a patient with a salivary gland tumor, positive for this mutation, that showed a excellent response to treatment with inhibitors of BRAF has been described [22].

We can speculate that the presence BRAF mutation may suggest combined biological thera-

peutic strategies to reduce the risk of relapse and increase of the life expectancy of SDC patients.

In conclusion, we believe that the molecular characterization of SDCs, after histo-morphological diagnosis, is essential for the choice of additional therapeutic options to integrate to classic chemotherapeutic regimens.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

SDC, salivary duct carcinomas; DBC, Ductal Breast Cancer; PET/CT, Positron Emission Tomography-Computed Tomography.

Author' contribution

MC and NSL designed the study and wrote the manuscript, FI and FL examined and treated surgically the patient, RDC, FF, GB established the diagnosis, GS performed immunohistochemical analysis and FC the gene mutational analysis. All authors contributed to the manuscript and approved the final version.

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