A secretory carcinoma with NTRK3 break-apart molecular rearrangement: A case report on a tumor initially diagnosed as a mucoepidermoid carcinoma.

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Introduction:

Although neoplasms of the salivary glands are relatively rare, they are nevertheless characterized by a variety of biologies and prognoses. According to the most recent WHO classification of salivary gland tumors, these are mostly benign but they can nevertheless present with different grades of malignancy. Secretory carcinoma (SC), a malignant tumor affecting the salivary glands of the head and neck, was recently reclassified in the light of molecular analysis in 2017; before that date it was considered a mammary analogue secretory carcinoma (MASC). Acinic cell carcinoma (ACC) is a low grade salivary gland malignancy characterized by serous acinar differentiation. It is important to understand the growth patterns and the differences between SC and ACC as they are similar histologically. Unlike ACC, SC pathognomonically expresses the ETV6-NTRK3 gene fusion, a characteristic permitting pathologists to formulate the diagnosis of a SC with regards to a lesion originally considered ACC. Although both SC and ACC are characterized by a generally favorable prognosis and non-aggressive behavior, high grade SC tumors have been described, and, according to some reports, they are characterized by a higher rate of lymph node involvement and distant metastases. Since it has only recently been reclassified, SC may be confused with other tumors of the salivary glands and incorrectly diagnosed. The current work, which reports on a case of SC of the submandibular gland originally diagnosed as a low grade mucoepidermoid carcinoma, examines some of its clinical, genetic and immunohistochemical features.

Case Presentation

A seventy-four year old female patient was examined at the outpatient section of the Maxillofacial Surgery Department of the University of Verona Medical Center for a submandibular lump on the right side. The lump was hard, mobile, and essentially asymptomatic. Other laterocervical lesions were not detectable at the time of palpation. The patient underwent both an ultrasonography of the neck, which detected a hypoechogenic lesion in the right submandibular gland, and a fine needle aspiration cytology (FNAC), which uncovered numerous epithelial cells exhibiting oval nuclei with moderate pleomorphism arranged in irregular, packed clusters, at times cruciform and papillary. There were also numerous cells with foamy cytoplasm and

eccentric nuclei; siderophages and isolated cells with cytoplasmic mucin vacuoles were less numerous. While there were some signs of mitosis, there were none of necrosis. All of these findings were compatible with a diagnosis of a low grade mucoepidermoid carcinoma.

The patient underwent a full body computed tomography (CT) (Figure 2) which uncovered a 6 mm (approximately) focal hypodense lesion of the right submandibular gland. The patient subsequently underwent total en bloc lesion resection and emptying of the right suprahyoid muscle.The postoperative course was uneventful. The histological analysis led to the diagnosis of a secretory carcinoma. The lymph nodes were cancer-free. The fluorescence in situ hybridazation (FISH) analysis using specific probes mapping the neurotrophic receptor tyrosine kinase (NTRK) NTRK-1, NTRK2 and NTRK3 chromosomal fusion regions identified a chromosomal rearrangement of the NTRK3 gene and the absence of a chromosomal rearrangment of mastermind Like Transcriptional Coactivator 2 (MAML-2) and Ewing sarcoma region-1 (EWSR1) genes. Histochemical analysis uncovered S100, 5D3, RN7, RCK108 protein and vimentin positivity. Routine postoperative test results were cancer-free and now, 12 months after the operation, the patient continues to be cancer-free (Figure 1a-b-c).

Discussion

Tumors of the salivary glands account for 3-4% of the tumors of the head and neck. The age group with the highest incidence is the one between 30 and 60 years with a peak in the 5th decade of life for benign forms and in the 6th decade for malign ones. The most frequently diagnosed malignant form is mucoepidermoid carcinoma. As mentioned earlier, initially cytological testing of the lesion led to a diagnosis of a mucoepidermoid carcinoma. SCs are considered to be very rare neoplasms. The typical presentation of SC, which tends to be uniform, is that of a slow-growing asymptomatic mass of the parotid or lateral cervical region. Despite its relatively uniform presentation, age at onset can vary significantly. The most common subsite for its presentation is that of the parotid gland, followed by the minor salivary glands and then the submandibular gland.

Just as most patients with lesions originating in the salivary glands, those with suspected SC normally undergo FNAC; but the test is frequently unable to lead to a correct diagnosis due to SC's rarity and of its relatively recent reclassification. Some studies have however shown that SC presents some well defined cytological features. It has generally been noted that the neoplasm has a papillary architecture and abundant, eosiphillic cytoplasm with focal oncocytic features and fine cytoplasmic vacuoles. Cells similar to myoepithelial ones are occasionally observed; the nuclei are moderately pleomorphic and charactrized by focal binucleations and irregular positioning. These characteristics are shared with other salivary gland types, and primarily with ACC.

Investigations endeavoring to distinguish between ACC and SC from a histological point of view are generally carried out in these cases. Periodic Acis-Schiff (PAS) staining, for example, is useful for guiding the diagnostic process. Its results help to differentiate between the two tumor types since ACC presents a more granular pigmentation. The molecular profiles of the two neoplasms differ as far as the S100 is concerned; it is in fact normally positive in SC and negative in ACC. With regard to DOG1, it is negative in SC and positive in ACC. But most importantly, the molecular analysis is decisive for identifying a SC since the ETV6-NTRK3 traslocation is virtually pathognomonic of SC, but it is never found in ACC. The presence of break-apart probes in >30% of neoplastic nuclei places the lesion within the group of NTRK3-rearranged tumors. The test result is characterized by a split in the NTRK3 signal (Bio-Optica probe). Red and green split fluorescent signals with a wild red/green signal at the side characterize the molecular abnormality in the diagnostic image (Leica Instrument). It is to be remembered that the FISH is a robust molecular cytogenic technology widely used in anatomical pathology laboratories in connection, for example, HER2+breast cancer, ROS1 and ALK rearranged lung cancers, 1p/19q in brain tumors, etc.

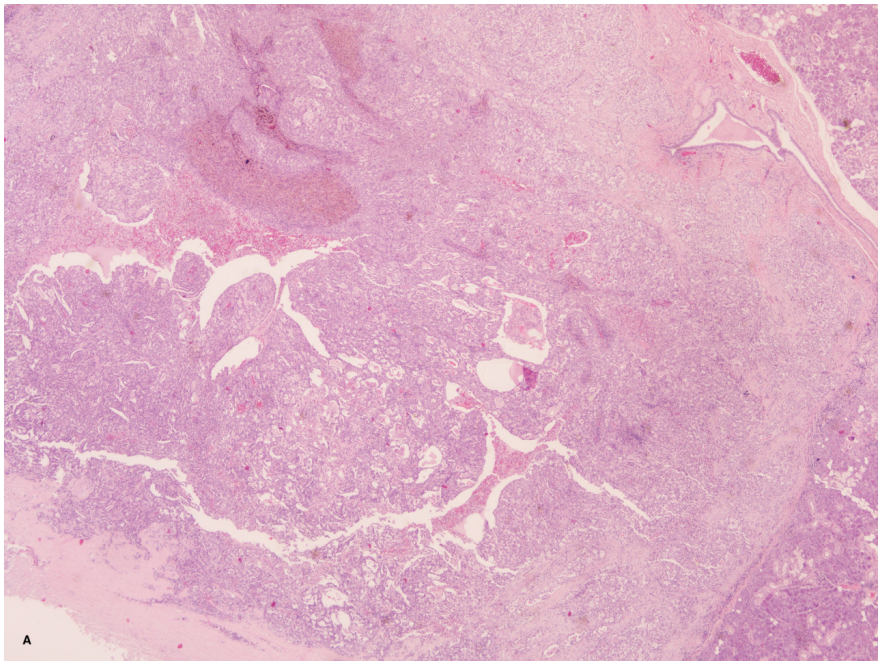
As far as patients' clinical status is concerned, SC seems to present a higher grade of malignancy at the time of diagnosis with respect to ACC and thus a worse disease free survival rate. Several case reports and case series studies have demonstrated that SC is more frequently chracterized by the development of lymph

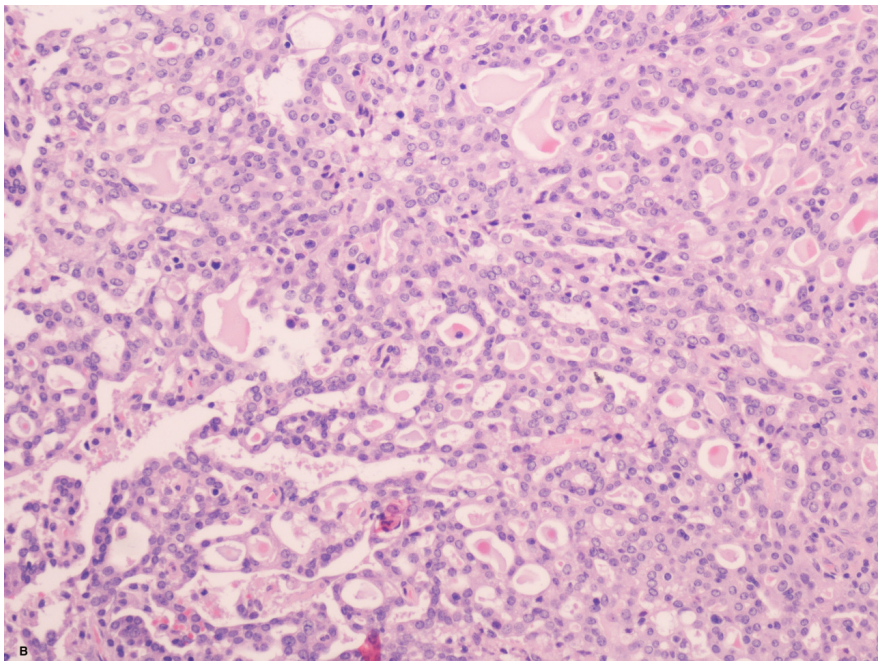
node involvement and distant metastasis with respect to ACC, and it has a higher rate of local recurrence. In view of the histotype's recent reclassification and the paucity of data that could lead to the formation of guidelines for its diagnosis and treatment, further research is urgent. Surgery has traditionally been the gold standard for the treatment of neoplasms of the salivary glands, followed by radiotherapy whenever evidence of aggressive histotypes, perineural and lymphovascular invasion or involvement of the seventh cranial nerve, the deep lobe, the resection margins or locoregional recurrence is identified. Chemotherapy is generally recommended in cases of recurrence or of distant localization of metastases. As far as SC is concerned, more lymph node involvement has been noted in patients with the characteristics mentioned above requiring the use of radiotherapy. According to some case series studies focusing on SC, some surgeons were not immediately in favor of treating the loco-regional lymph node recurrence. For the most part, however, in those case the patients underwent adjuvant radiotherapy or laterocervical emptying. The only case of recurrence described in the literature occurred in a patient who did not undergo adjuvant radiotherapy. In the light of that report and of the higher incidence of lymph node positivity in SC with respect to that in ACC, the degree of tumor aggressiveness of the former appears to be greater and more comparable to that of a mucoepidermoid carcinoma than to that of ACC.

Conclusion

The real prevalence of SC is probably underestimated given the recent histological reclassification of salivary gland tumors, and molecular profiling testing is generally recommended. As no clear cut morphological signs characterizing this neoplasm have as yet been identified, FISH analysis with the NTRK3 probe is recommended when there are morphological signs pointing to the hypothesis of a differential diagnosis. The FISH technique could alternatively be carried out whenever the morphology of the lesion could be indicative of a mucoepidermoid or an ACC carcinoma. The biological behavior of the former can present a variety of characteristics but its high grade of aggressiveness with respect to the latter leads us to suggest that it is best to carry out elective treatment of the lymph nodes of the neck and adjuvant radiotherapy in situations of greater risk.

Figure 1: Secretory carcinoma . **A** - At low power, secretory carcinoma (left side) appears as a nodule with pushing and well defined borders toward the normal salivary gland (right side) (Hematoxylin&Eosin, 10X). **B-** at higher magnification, the microhistological architecture is appreciable as numerous microcysts bordered by plump epithelial cells with intraluminal secretion (Hematoxylin&Eosin, 40X). **C-** The secretory material in the microcyst lumen is highlighted by PAS (Periodic Acid Shift) diastase digestion resistant staining (PAS-D, 40X)





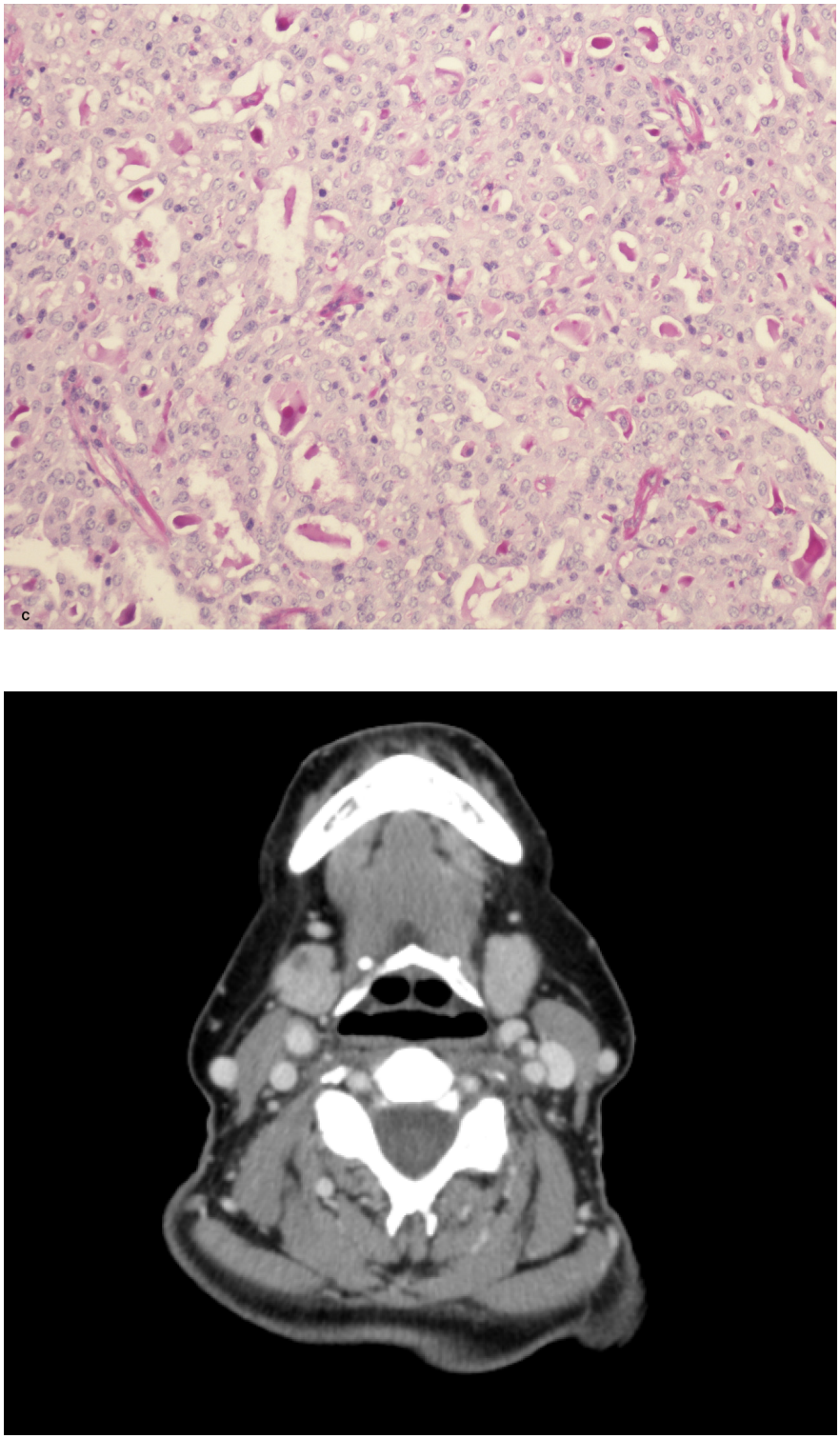


Figure 2: CT scan , axial view: the lesion is detectable in the context of the right submandibular gland.

Bibliography