

Use of Next-Generation Sequencing Techniques in the Accurate Diagnosis of Neural Crest Cell-Derived Tumors

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Abstract

The increasing adoption of next-generation sequencing (NGS) techniques in daily practice has led to the incorporation of molecular information in the diagnosis and management of cancer patients. Taking advantage of these new tools could clearly impact the outcome of cases we present the case of a 39-year-old woman who attended the emergency department for fever and dysphagia. Computed axial tomography revealed a solid lesion in the subcarinal region. Histologically, the mass showed a tumor with proliferation of spindle cells containing elongated, hyperchromatic nuclei and brownish pigment in the cytoplasm. On immunohistochemical staining, the cells were positive for S-100, SOX10, P16 and HMB 45 and negative for CKAE1/AE3 and actin. Consequently, given the location and clinical findings, the mass was tentatively diagnosed as locally advanced esophageal mucosal melanoma. Further genetic testing with an NGS panel identified EWSR1-ATF1 fusion, resulting from translocation t(12;22)(q13-14;Q12). These new data were combined with all the available information, and the patient was diagnosed with clear cell sarcoma, which was treated with radical-intent radiotherapy. The use of NGS platforms to determine the genetic profile of the tumor facilitated the correct diagnosis of this rare neoplasm with melanocytic differentiation, an entity that presents a broad differential diagnosis with other neural crest cell-derived tumors. As a result, the patient received proper treatment based in precision medicine in line with the new molecular findings described.

Keywords: Clear Cell Sarcoma; Next-Generation Sequencing; EWSR1-ATF1 Fusion, Neural Crest

Introduction

Histological examination together with immunohistochemistry (IHC) techniques is currently the gold standard in the diagnosis of cancer [1]. Next-generation sequencing (NGS) techniques offer several clinical benefits, one of which is to guide the diagnosis of tumors that harbor specific molecular alterations [2]. Thus, a single entity, that years ago was characterized on the basis of its morphology, can now be sub-classified in much more detail into distinct molecular entities genetically defined by the presence of a specific molecular alteration [2]. This way, techniques based on the identification of somatic genetic variants and machine learning analysis have been able to correctly classify cancer samples according to their primary site [3].

The data generated from these molecular analyses can help diagnose cancers with higher precision, especially in cases that are difficult to characterize. The

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detection of the BRAF gene mutation in a thyroid aspirate suggestive of malignancy [4], the identification of malignancy in cytological samples [5], and the classification of tumors exhibiting undefined morphologies, such as sarcomas or undifferentiated tumors [6], have all been reported. In current routine clinical practice, it falls to the pathologist to integrate and interpret morphological and molecular information in order to resolve diagnostic, prognostic and predictive questions [7].

Clinical Case

We present the case of a 39-year-old woman with a history of mediastinal B-cell lymphoma with sclerosis diagnosed in 2009, who was treated with chemotherapy and autologous bone marrow transplant. At the time of the visit, she had achieved a complete response. The patient attended the emergency department for fever and dysphagia. Computed tomography (CT) revealed a solid 34 mm × 56 mm lesion in the subcarinal region. Carinal stenosis was observed on fiberoptic bronchoscopy. Fine needle aspiration of the area was carried out, but insufficient cytological material was obtained to make a diagnosis. Gastroscopy also showed a submucosal tumor 24 cm from the dental arch; positron emission tomography (PET-CT) reported no findings of interest except for a mediastinal mass suggestive of malignancy, with a standard uptake value (SUV) of 18.09 (Figure 1 A-D), and no signs of spread to other levels. Additionally, brain magnetic resonance imaging and hematological examination of the bone marrow and peripheral blood showed no abnormalities. Finally, a surgical biopsy was performed, where a tumor mass was seen to have invaded the esophagus and confirmed that it was unresectable.

The anatomopathological study showed that the tumor lesion was composed of spindle cells arranged in a diffuse storiform pattern. The tumor cells showed a hyperchromatic elongated nucleus, with eosinophilic nucleoli and the occasional presence of brownish pigment in the cytoplasm (Fig. 2, panel A). IHC staining was positive for S-100, SOX10, P16 and HMB 45 (Fig. 2, panels B, C and D) and negative for CKAE1/AE3 and actin. In view of these findings, the tumor was tentatively diagnosed as a malignant melanocytic lesion.

In order to characterize the lesion, the study was completed with an NGS panel (Foundation One® CDX, Roche) [8]. This analysis showed the presence of EWSR1-ATF1 fusion, resulting from translocation t(12;22)(q13-14;q12). Other findings are shown in Table 1.

This fusion is characteristically found in clear cell sarcomas (CCS), which are soft tissue tumors with melanocytic differentiation, found exceptionally in the anatomic region described in our case. At present, the patient has received radiotherapy and is awaiting re-evaluation of response.

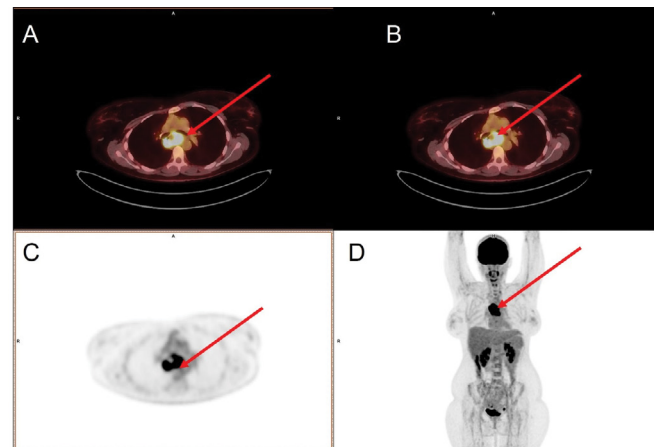


Figure 1: Transversal (A-C) and longitudinal (D) positron emission tomography computed tomography (PET-CT) images showing a mediastinal mass suggestive of malignancy (red arrows).

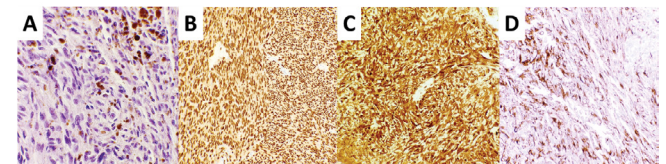


Figure 2: Histological staining of patient specimens: A) hematoxylin-eosin, B) SOX-10, C) P16 and D) HMB 45.

Table 1: Summary of the genetic results.

Pathogenic variants	EWSR1-ATF1
Variants of uncertain significance	MCL1 E110 del
TMB	0
MSI	stable

MSI, microsatellite instability; TMB, tumor mutational burden (mutations/Mb)

Discussion

CCSs, first described in 1965 by Enzinger et al. [9], are rare, aggressive tumors that account for less than 1% of soft tissue sarcomas [10]. They usually affect young adults and are located in 95% of cases in the lower extremities, primarily in the foot and ankle, where they tend to present as a slow-growing indolent mass in the soft tissue [11, 12]. When they are found in the abdominal cavity, the main symptoms are abdominal pain, weight loss, anemia, lethargy, or pyrexia and, occasionally, vomiting or hematemesis [10, 13]. Their average size is 4.5 cm, with a range of 2.4 to 15 cm [14]. Diagnosis is complex, since they can be easily confused with malignant melanomas due to their identical IHC and ultrastructural characteristics [14-16].

These types of tumors have a high rate of relapse and metastasis, with survival rates of 50% to 62.9% at 5 years, and 38% to 51.3% at 10 years [17, 18]. Unlike those with

advanced disease, patients with localized disease and tumors ≤ 3 cm have a better prognosis [18].

Among the findings that guide differential diagnosis, the presence of a low mitotic index and mild-moderate pleomorphism is more indicative of CCS [15]. The only way to make a correct diagnosis is to detect the presence of EWSR1-ATF1 fusion, since both lesions show an identical immunophenotype regarding IHC staining [15]. This fusion can be detected by techniques such as fluorescent in situ hybridization [19], or, as in our case, by NGS.

Both the identification of the less common translocation t(2;22)(q33;q12) and the characterization of tumors containing the translocation t(12;22)(q13;q12) have helped to distinguish this tumor entity [19, 20]. This translocation leads to expression of the EWSR1-ATF1 fusion protein that is characteristic of CCS and confers a series of traits associated with the pathophysiological mechanism of the disease, since it regulates oncogenic and differentiation programs in this entity [21]. According to the information provided by both cell and animal models, the EWSR1-ATF1 alteration seems to be the driver alteration in this disease [22-24]. Furthermore, a recent study has revealed that this fusion causes the cells of these tumors to activate the proliferation and differentiation programs typical of CCS, mechanisms they share with malignant melanomas [21].

The activity of this fusion protein is related to the activation of transcription factors TFAP2, SOX10 and MITF, described as mediators in the initial stages of neural crest induction, specification, migration, and differentiation [25]. Alterations in their expression are associated with the metastatic behavior of neural crest cell-derived tumors [26, 27], a characteristic that indicates the neuroendodermal origin of the cells of these tumors and enables them to differentiate into a melanocytic lineage [28, 29].

NGS techniques can detect new clinically relevant genetic alterations, for example in the case of leukemia [30] or lung cancer [31], opening the door to more personalized treatments. In our case, detection of the EWSR1-ATF1 translocation led to a more precise patient's diagnosis and a therapeutic switch to radical-intent radiotherapy. Nevertheless, the limitations of NGS techniques should also be taken into account. One of these is the heterogeneity of tumor samples, whether static (in the same tumor) or dynamic (over time), and which may lead to discrepancies in the results obtained with these techniques [32]. Another limitation of NGS is that it can detect cancer-associated mutations in healthy tissues [33]. These genetic variants, which appear as a result of aging in healthy tissue, do not have the same impact as in tumor tissue [34].

Conclusions

NGS molecular characterization of this case facilitated

the correct diagnosis of this rare tumor subtype allowing the differentiation with other tumor types of the neural crest lineage. Thanks to the results obtained using NGS, a precise diagnosis was reached that allowed a better tumor characterization and a more adequate treatment regime.

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Conflict of Interest

Jesús García Donas declares having received research funding and speaker fees from Roche. The rest of the authors declares no conflict of interest.

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