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NUT carcinoma in a nutshell: A diagnosis to be considered more frequently

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ABSTRACT

NUT carcinoma is a rarely diagnosed, poorly differentiated subtype of squamous cell carcinoma, defined by chromosomal rearrangements of the gene encoding nuclear protein of the testis (NUT). It is characterized by extremely aggressive clinical behavior resulting in a dismal prognosis, with a median survival of 6.7 months. Though most frequently detected along the body midline, NUT carcinoma can arise in any organ. Fewer than 100 cases have been reported in medical literature with the majority of patients being children or young adults. Here we present a case of sinonasal NUT in a 48-year-old male who came to our hospital due to progressive cephalalgia. Radiographically, an irregular mass in the left sphenoidal sinus suspicious for a malignant process was detected, and biopsies were taken. Histopathologically, a tumor of highly mitotic, predominantly small to middle-sized cells with a focal abrupt transition to mature-appearing, squamous epithelium was noted. Of critical importance for the diagnosis, the undifferentiated tumor cell population robustly expressed NUT. The diagnosis of NUT carcinoma was confirmed by the identification of BRD4-NUT fusion. This case integrates typical morphological, immunohistochemical and molecular characteristics of NUT carcinoma and highlights the need to consider this entity in cases of poorly differentiated squamous carcinoma.

1. Introduction

NUT carcinoma is an extremely rare and lethal subtype of poorly differentiated squamous carcinoma, genetically defined by a fusion rearrangement that involves the gene nuclear protein of the testis (NUT)[1] . Most patients present at a young age, with a rapidly progressing tumor of the head and neck region or mediastinum [1]. To date, owing to the absence of effective treatments, NUT carcinoma confers an extremely poor prognosis with a median survival of less than 7 months [2]. Clinically, the diagnosis is most frequently established immunohistochemically by using a monoclonal NUT-directed specific antibody. Here we report clinical, pathological and molecular characteristics of a sinonasal case of NUT carcinoma in an uncommonly old male that initially presented with severe headaches and was found to have a rapidly growing mass in the left sphenoidal sinus.

2. Materials and methods

For gene fusion detection analysis, targeted RNA sequencing was conducted. Tumor tissue from formalin-fixed, paraffin-embedded material was morphologically enriched to a percentage of 30% tumor nuclei followed by RNA isolation. A targeted RNA fusion panel (Archer® FusionPlex® Solid Tumor Panel, ArcherDX, CO, USA) was applied to prepare target-enriched cDNA libraries from the RNA using anchored multiplex polymerase chain reactions and unidirectional gene-specific primers. Final targeted amplicons were sequenced on an Ion Torrent™ Ion S5™ XL next-generation sequencing platform (ThermoFisher Scientific, MA, USA). As such, fusions and other mutations of a number of 53 cancer-associated genes (including NUT) were covered: AKT3, ALK, ARHGAP26, AXL, BRAF (fusion and V600E mutation), BRD3, BRD4, EGFR, ERG, ESR1, ETV1, ETV4, ETV5, ETV6, EWSR1, FGFR1, FGFR2, FGFR3, FGR, INSR, MAML2, MAST, MAST2, MET, MSMB, MUSK, MYB, NOTCH1, NOTCH2, NRG1, NTRK1, NTRK2, NTRK3, NUMBL, NUT, PDGFRA (fusion and mutation), PDGFRB, PIK3CA, PKN1, PPARG, PRKCA, PRKCB, RAF1, RELA, RET, ROS1, RSPO2, RSPO3, TERT, TFE3, TFEB, THADA and TMPRSS2.

3. Case report

A 48-year-old male presented to our University Hospital with progressive cephalalgia for more than two weeks, aggravating at late daytime. Cranial computed tomography and magnetic resonance imaging showed a soft tissue mass in the left sphenoidal sinus (longest diameter of 4.7 cm) involving the surrounding bone. In the week prior

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[1] NUT; nuclear protein of the testis.

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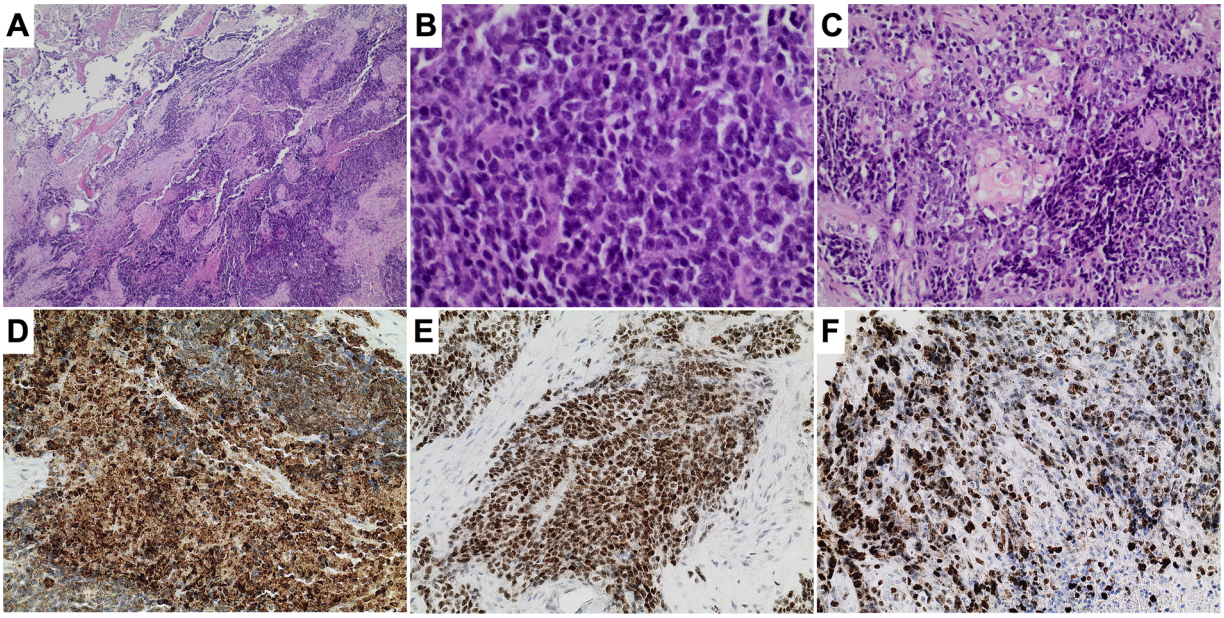

Fig. 1. Morphological and immunohistochemical features of NUT carcinoma.
A: Sinonasal mucosa and bone tissue were infiltrated by a partially necrotic tumor. B: The tumor was predominantly composed of small to middle-sized cells with scant, glycogen-rich cytoplasm and hyperchromatic nuclei. C: Adjacently, focal nests of mature-appearing squamous cells with abortive keratinization were noted. D: The tumor cells were homogenously positive for AE1/3. E: The tumor cells showed a strong nuclear signal for NUT. F: Ki-67 proliferation activity reached 60%. Original magnifications: 40x [A], 400x [B], 200x [C, D, E, F].

to surgical excision, the patient developed oculomotor nerve palsy with consequent double vision, exophthalmus and ptosis. During surgery, at the base of the left sphenoidal sinus a white, irregularly shaped mass was noted, biopsied and sent to our department for pathological evaluation. Histologically, connective and bone tissue were infiltrated by a partially necrotic tumor with high mitotic activity, composed of relatively monomorphic, small to middle-sized tumor cells with scant, glycogen-rich cytoplasm and hyperchromatic nuclei containing prominent nucleoli (Fig. 1A-B). In some areas, an abrupt transition of these cells to a morphologically different cell population in the form of nests of mature appearing, squamous epithelium with pale eosinophilic cytoplasm was noted (Fig. 1C). Abortive keratinization was noted focally, however no pearl formation was detected. As evinced by immunohistochemistry, the tumor cells homogenously and robustly expressed AE1/3, CK5/6 and p40 (Fig. 1D). Of note, the undifferentiated tumor cell population exhibited strong and uniform nuclear immunoreactivity for NUT (Fig. 1E), while the expression in the more mature component was only weak. The proliferation activity as determined by the Ki67-proliferation index reached 60% in hot spot areas (Fig. 1F). Reactivity for synaptophysin and p16 was partial and mostly weak, while no signal was found for chromogranin A, desmin, S100 and LMP of Epstein-Barr-Virus. As such, in synopsis of the morphological and immunohistochemical findings, the diagnosis of a sinonasal NUT carcinoma was established. The diagnosis was confirmed molecularly by identification of BRD4-NUT fusion, with breakpoints in the BRD4 exon 11 (NM_014299) and the NUT exon 2 (NM_175741) in more than 1300 reads using next-generation sequencing technology. After assessment of the diagnosis, the patient underwent tumorreductive surgery followed by radiochemotherapy. At the date of last contact, six months after initial diagnosis, the patient was in a stable physical condition (Karnofsky performance status score = 70%), accompanied by significant radiographic evidence of tumor regression post radiochemotherapy.

4. Discussion

NUT carcinoma is a rare, genetically defined, highly aggressive tumor defined by chromosomal rearrangement of the gene NUT, resulting in different fusion products, most often in the form of BRD4-NUT. The entity was first described in 1991 by Kubonishi et al. in a patient in retrospect mistakenly classified as thymic carcinoma [3]. Recognition of NUT carcinoma as a distinct clinicopathological entity was in fact established more than ten years later by French et al. [4,5]. Morphologically, an abrupt transition of immature cells juxtaposed to well-differentiated, mature appearing squamous nests is considered the only distinct feature. Due to the lack of pathognomonic morphological characteristics, its rarity and only recent description, diagnosis of NUT carcinoma still is a challenge for pathologists and probably diagnosis is made too infrequently [6]. The actual challenge, however, is not the diagnosis itself, which can easily be established by immunohistochemistry using the NUT-specific antibody, but the question of when to consider NUT carcinoma, particularly since virtually every organ can be affected. Even the initially presumed characteristic midline location of these tumors turned out to be a misleading trait, as a number of tumors appear at lateral sites. The term “midline” was consequently removed from the entity’s name in the current World Health Organization Classification of Head and Neck Tumours. Another factor contributing to misdiagnosis of NUT carcinoma is the assumption that NUT carcinoma is restricted to patients of childhood or young adulthood. However, today it is clear that though there is a pediatric-weighted age distribution, NUT carcinoma can occur in patients of all ages, including older adults, as exemplified in the presented case [7]. Still, nothing is known about the etiology of NUT carcinoma, though smoking as a major risk factor for conventional squamous carcinoma is very unlikely to play a pathogenic role in NUT carcinoma according to epidemiological data and given the described age distribution [8]. On the contrary, diagnosis of a poorly or undifferentiated carcinoma in a never-smoking individual of particular young age should prompt clinicians to consider NUT carcinoma. Also in contrast to conventional squamous carcinoma, in NUT carcinoma no association with Epstein-Barr virus or human papilloma virus infection has been demonstrated so far [7].

Due to its aggressive nature, rapid proliferation and early metastasis, NUT carcinoma is associated with a dismal prognosis, with a

median survival of only 6.7 months. Though to date there is no established treatment protocol, recent retrospective analyses indicate that initial surgical resection with or without adjuvant chemoradiation can prolong overall survival rates, while radiation or chemotherapy alone were not beneficial [9]. Current trials evaluate the effect of new targeted therapies including bromodomain and histonedeacetylase inhibitors, which may exhibit therapeutic potential, according to recent reports [10]. Indisputably, a thorough molecular analysis of a large series of NUT carcinomas is urgently needed to identify other specific therapeutic targets that drive the rapid progress of this tumor.

Declaration of conflicting interests

The authors declare that there is no conflict of interest regarding the publication of this article.

Informed consent

The use of tissue specimens was approved by the University's ethics committee (approval code 206/2005). The reported patient gave written informed consent upon therapy initiation.

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