NUT Carcinoma of the Sublingual Gland

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Received: 29 September 2015 / Accepted: 13 November 2015 / Published online: 19 November 2015
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Abstract NUT carcinoma (NC) is a recently described, rare and extremely aggressive cancer primarily located to supradiaphragmatic structures and affecting young individuals. NC is characterized by translocations involving the *NUT* gene on 15q14 with the most common translocation partner gene being *BRD4* on 19p13, resulting in the t(15;19)(q14;p13) karyotype. NC is poorly differentiated and is likely to be overlooked and misdiagnosed as poorly differentiated squamous cell carcinoma (SCC) when immunohistochemical evaluation of NUT protein expression is omitted. Previously, NC has been found in the parotid and submandibular glands and we present the first case in the sublingual gland arising in a 40-year-old woman. We discuss the diagnostic considerations for poorly differentiated carcinomas of the salivary glands and advocate the inclusion of NUT immunohistochemistry in this setting. Not only does the NC diagnosis confer a grave prognosis when treated as SCC as illustrated by the present

case, but is important for the inclusion of patients in ongoing clinical trials.

Keywords Salivary gland · Carcinoma · Sublingual gland · NUT · BRD4-NUT · Fusion protein

Introduction

Recently, a rare and aggressive type of carcinoma has been described, mainly located to supradiaphragmatic midline structures, especially in the head and neck and mediastinum [1]. The carcinoma is characterized by translocations involving the *NUT* gene (aka *NUTM1*) on chromosome 15q14 and various partner genes; two-thirds of these involve the *BRD4* gene resulting in a t(15;19)(q14;p13.1) karyotype [2]. The tumor is biologically driven by overexpression of *NUT*-fusion genes, which block differentiation and maintain tumor growth [3]. Accordingly, the carcinoma has been named NUT midline carcinoma. Because an increasing number of cases have been reported outside the midline, the World Health Organization has changed the name to NUT carcinoma (NC) [4].

Clinically, the patients are characterized by their young age and a rapidly fatal clinical course with a median survival time of 6.7 months [5, 6]. Subsequent studies have revealed NC to constitute 18 % of Epstein–Barr Virus (EBV) negative undifferentiated carcinomas of the upper aerodigestive tract, making it a likely under diagnosed tumor entity of the head and neck [7]. Histopathologically, NC is an epithelial neoplasm composed of poorly differentiated basaloid cells with focal squamous differentiation and immunohistochemically positive for cytokeratin 7 (CK7) and p63 [8]. To date only one NC of the submandibular gland and one of the parotid gland has been

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reported [9, 10]. In this paper, we report a rare case of NC outside the midline in the head and neck and the first to arise in the sublingual gland.

Case Report

A 40-year-old female with no significant medical history was referred with complaints of swelling and meal-related pain in the right sublingual region for the past month. Clinical examination revealed a mobile $4 \times 4 \times 2$ cm mass in the right sublingual area covered with intact mucosa. The neck was unremarkable. Subsequent CT-scan identified a well circumscribed contrast enhancing sublingual gland mass with a necrotic center without lymph node involvement, staged as T2N0M0 [11]. Intraoperative frozen section showed carcinoma and the tumor was resected down to muscle and bone along with the Wharton duct and lingual nerve. Surgery was macroscopically radical but the margins were microscopically involved. Final histology showed poorly differentiated squamous cell carcinoma (SCC) with basaloid features. One month later the patient presented with an enlarged lymph node in level II on the right side of the neck, pain in right hypochondrium and left hip. Fine-needle aspiration cytology from the neck showed SCC but before commencement of radiotherapy (RT), the patient was readmitted due to lumbar pain. Repeated CT-scan revealed osteolytic foci in the sacrum and both iliac bones and in the cervical, thoracic, and lumbar vertebral column with epidural growth in Th8–11 (Fig. 1). Palliative RT at Th7–11 was administered to prevent spinal cord compression. Gynecological and gastrointestinal examinations were normal. Three and a half months after diagnosis, cervical spinal cord compression evolved for which regional RT was initiated. A Paclitaxel and Capecitabine regimen was attempted without response. Metastases to the right humerus, left clavicle, and femoral column resulting in a fracture in the latter subsequently developed. The patient died 5½ months after presentation.

Materials and Methods

Histopathology and Immunohistochemistry

The specimen was formalin-fixed and paraffin-embedded, then stained with haematoxylin and eosin. Immunohistochemistry was performed on the Ventana Benchmark Ultra platform (Ventana Medical Systems, Tucson, AZ). The following antibodies were used: Ki-67 (clone MIB-1, code M724001, mouse anti-human, 1:100, Dako, Glostrup, Denmark), CK7 (clone OV-TL 12/30, code M701801, mouse anti-human, 1:1000, Dako), cytokeratin 5/6 (CK5/6) (clone D5/16 B4, code M723701, mouse anti-human, 1:20,

Fig. 1 CT-scan of the axial skeleton. CT-scan demonstrated tumor masses in Th8 and Th11. Metastases to C7 along with sacral and both iliac bones were also found in the same scan (not shown)

Dako), cytokeratin 20 (CK20)(clone KS20.8, code M701901, mouse anti-human, 1:400, Dako), p63 (clone 4A4, code 790-4509, mouse anti-human, ready-to-use, Roche, Hvidovre, Denmark), S100 (polyclonal, code 20311, rabbit anti-human, 1:4000, Dako), CD56 (clone 1B6, code NCL-CD56-1B6, mouse anti-human, 1:50, Novocastra, Ballerup, Denmark), synaptophysin (clone, MRQ-50, code 760-4595, rabbit anti-human, 1:150, Roche), chromogranin A (polyclonal, code A043001, rabbit anti-human, 1:2000, Dako), CD117 (polyclonal, code A450229, rabbit anti-human, 1:100, Dako), TTF-1 (clone SPT24, code NCL-TTF-1, mouse anti-human, 1:100, Novocastra), HER2 (clone 4B5, code 790-2991, rabbit anti-human, ready-to-use, Roche), p16 (clone E6H4, code 825-4714, mouse-anti-human, ready-to-use, Roche) and NUT (clone C52B1, code 3625, rabbit anti-human, 1:50, Cell Signaling Technology, Danvers, MA). Diluent (code S0809, Dako) was used. Appropriate positive controls were used and negative control sections were incubated identically except for the primary antibody, which was replaced by normal rabbit serum/mouse IgG.

The Epstein–Barr Virus (EBER) PNA Probe/Fluorescein kit was used (Dako, code Y5200) and visualized with

the Dako PNA ISH Detection Kit (Dako, code K5201). Four-micrometer-thick paraffin sections were deparaffinized, rehydrated, and processed according to the manufacturer's instructions.

Fluorescence In Situ Hybridization (FISH)

BRD4 and *NUT* dual color split-apart FISH was performed as described using *NUT* 5' centromeric BAC probes RP11-368L15 and RP11-1084A12 (biotin-labeled, red), and 3' telomeric probes, RP11-1H8 and RP11-64o3 (digoxigenin labeled, green) [12]. *BRD4* 5' centromeric probes were RP11-207i16 and RP11-3055m5 (biotin labeled, red), and 3' telomeric probes were RP11-319o10 and RP11-681d10 (digoxigenin-labeled, green).

Results

Histopathology, Immunohistochemistry, and In Situ Hybridization

Gross

The resected specimen was 40 × 35 × 15 mm, firm with a heterogeneous yellowish-red colored cut surface with dispersed necrotic areas. There was no identifiable normal salivary gland.

Histopathology

The tumor had no connection to the overlying normal squamous epithelium. In some areas the carcinoma cells followed the lobular architecture of the gland, but frequently invaded beyond these and infiltrated duct and acinar cells of surrounding normal sublingual gland and into the surrounding connective tissue and muscle. Tumor cells were predominantly composed of confluent groups of poorly differentiated small and medium sized cells with vesicular nucleoli (Fig. 2a). Small foci with abrupt squamous differentiation with cytoplasmic keratohyalin granules were noted. All tumor cells were positive for NUT and p63 (Fig. 2b, c). CK7 (Fig. 2d) and CK5/6 had similar staining patterns with >80 % of the poorly differentiated component and 100 % of the squamous component being positive, most intensely in the latter. Synaptophysin was positive in the cytoplasm of less than 1 % of the poorly differentiated component and negative in the squamous component. Ki-67 index was 80 %. The immunohistochemical stainings for CK20, CD56, chromogranin A,

S100, CD117, TTF-1, HER2, and p16 along with in situ hybridization for EBER were negative.

FISH

Dual color split-apart FISH revealed split apart of both *NUT* and *BRD4* red and green flanking probes, consistent with *BRD4-NUT* fusion (Fig. 3a, b).

Discussion

The diagnosis of NC is challenging, especially in the salivary glands as these harbor numerous morphologic mimics. The main consideration is poorly differentiated SCC, but also lymphoepithelial carcinoma and large-cell neuroendocrine carcinoma are entities with histopathologic overlap [13, 14]. The two latter can be excluded with EBER in situ hybridization and several neuroendocrine markers (CD56, chromogranin A, synaptophysin), respectively. Once these are excluded, the diagnosis of poorly differentiated SCC is appropriate. NC is a subtype of SCC, and therefore must be excluded in this setting. In the present case, NC was not diagnosed until it was reviewed in connection with another study on our institutional material where suspicion of NC was raised. The diagnosis of NC can be made by demonstration of diffuse (>50 %) nuclear staining for NUT by immunohistochemistry using the commercially available C52 monoclonal antibody (Cell Signaling Technologies, Danvers, MA). This antibody has a sensitivity of 87 % and specificity of 100 %, and is therefore considered diagnostic of NC, although two immunohistochemically positive cases with intact split apart FISH signals in the upper respiratory tract has been reported [15, 16]. Confirmation by FISH is not necessary, but can help determine the fusion partner to *NUT*. *BRD4* is the most frequent translocation partner of the *NUT* gene, involved in about two-thirds of NCs, but several other genes, including *BRD3* and *NSD3* have been shown to serve as fusion partners with *NUT* in NC; these are termed *NUT*-variants [3, 12]. It is not certain whether the variant fusions confer different prognoses than that of *BRD4-NUT*, however evidence suggests similar sensitivity to targeted inhibitors of *BRD4*, termed BET inhibitors, for which early clinical trials are enrolling NC patients (clinicaltrials.gov Identifiers: NCT01587703, NCT01987362, NCT02259114, NCT02259114, NCT02158858). Poorly differentiated SCC is a high-grade salivary gland carcinoma, but has a better prognosis than NC and therefore the distinction between these is important. With this report NC is described in all the major salivary glands, and we therefore advocate the inclusion of NUT immunohistochemistry in poorly differentiated carcinomas of salivary gland origin. This adds NC to the

Fig. 2 Hematoxylin and eosin (HE) stain and immunohistochemical stain of resected specimen. **a** HE showing undifferentiated carcinoma with basaloid appearance and areas of eosinophilic cells with distinct cell borders, consistent with squamous differentiation. **b** NUT staining showing characteristic speckled, nuclear reaction. Normal

sublingual gland in the top of the picture. **c** Nuclear p63 staining, colocalizing with NUT. Myoepithelial and basal cells in the normal sublingual gland are also stained. **d** Cytokeratin-7 was observed in the cytoplasm of most carcinoma cells along with the normal sublingual gland ducts. Scale bar = 100 μ m

Fig. 3 Dual color, split-apart fluorescence in situ hybridization (FISH) for *NUT* and *BRD4* gene status. FISH showed separated telomeric (green) and centromeric (red) signals for probes flanking the *NUT* (a) and *BRD4* (b) genes, respectively, consistent with *BRD4-NUT* gene fusion

list of salivary gland carcinomas driven by fusion oncogenes, with diagnostic and therapeutic implications [17, 18].

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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