EBV-Positive Plasmacytoma of the Submandibular Gland— Report of a Rare Case with Molecular Genetic Characterization

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Abstract Plasmacytomas are differentiated plasma cell tumors that present as a mass lesion in osseous or extra-osseous sites. Although the most common site for extramedullary plasmacytomas (EMP) is in the upper respiratory tract, plasmacytomas initially presenting as salivary gland masses are very uncommon. We describe a case of an EBV-positive plasmacytoma presenting as a 7.7 cm submandibular mass in an elderly immunocompetent man which displayed an abundance of “naked nuclei” on fine needle aspiration cytology. The tumor showed lambda light chain restriction and positive expression for CD38, MUM1 and EBER. Subsequent investigation for myeloma revealed absence of M-protein and end-organ damage, except for a lytic lesion in the radial bone. An extensive fluorescent in situ hybridization analysis showed the tumor to be negative for the t(4;14) *FGFR3/IGH* translocation as well as translocations involving the *IGH*, *IGL*, *IGK*, *CCND1*, *BCL2*, *BCL6* and *C-MYC* genes. *KRAS* genetic analysis did not reveal any mutations of codons 12, 13 and 61.

Keywords Plasmacytoma · Salivary gland · Epstein barr virus · Fluorescence in situ hybridization · *CMYC* · *KRAS*

Introduction

Plasmacytomas are differentiated plasma cell neoplasms that present as localized lesions either in the bone (osseous plasmacytoma) or non-osseous sites (extramedullary plasmacytoma; EMP). Although the most common site for EMP is in the upper respiratory tract, plasmacytomas initially presenting as salivary gland masses are very uncommon. We describe the clinicopathological features of a case of an Epstein-Barr virus (EBV)-positive plasmacytoma presenting as a large submandibular mass in an elderly immunocompetent man.

Materials and Methods

The specimen was fixed in formalin, embedded in paraffin and 4 µm thin sections were cut and stained with Hematoxylin and Eosin. An immunohistochemical study was performed using CD3, CD5, CD20, CD38, CD43, CD45, CD56, CD79a, CD138, MUM1, Ki67, cytokeratins (AE1/3), epithelial membrane antigen (EMA) (DAKO A/S, Glostrup, Denmark) and cyclin D1 (Thermo Fisher Scientific, CA, USA) according to the manufacturer’s instructions. In situ hybridization for EBV-encoded small RNAs [EBER], kappa and lambda light chains (Leica Microsystems, Newcastle upon Tyne, UK) was also performed.

Fluorescent in situ hybridization (FISH) was done on 4 µm thin sections using split-signal DNA probes specific for 8q24 (*C-MYC*), 18q21 (*BCL2*), 3q27 (*BCL6*), 11q13 (*CCND1*), 22q11 (*IGL*), 2p12 (*IGK*) and 14q32 (*IGH*), and dual-fusion DNA probes specific for the t(4;14) *FGFR3/MMSET* and *IGH* translocation (Abbott Molecular, Illinois, USA). Fluorescence in situ hybridisation was performed using the DAKO FISH ancillary kit (DAKO A/S, Glostrup,

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Denmark) according to the manufacturer's instructions. Briefly, the slides were de-waxed in xylene and rehydrated through decreasing concentrations of alcohol. They were then heated in a microwave oven (10 min, 750 W) in pre-treatment buffer and then subjected to pepsin digestion for 3 min at 37°C. After a wash, they were dehydrated in increasing concentrations of alcohol and allowed to air dry. The hybridisation mixture containing FISH probe, water and hybridisation buffer (in 1:2:7 ratio) was then applied, cover-slipped and sealed with vulcanising rubber. When the rubber cement had dried, slides were placed in a hybridiser (DAKO) and denatured at 82°C for 5 min, followed by overnight hybridisation at 37°C. The next day, the slides were washed in stringency buffer at 65°C for 10 min and rinsed in wash buffer twice for 3 min. Slides were mounted using Vectorshield fluorescence mounting medium containing DAPI (Vector Laboratories Inc, Burlingame, California, USA) and viewed using a fluorescence microscope equipped with a dual pass fluorescein isothiocyanate/rhodamine filter and an ultraviolet longpass filter (Chroma Technology Corp, Rockingham, Vermont, USA). The cut-off values for the interphase FISH analyses were established according to the criteria of Ventura et al. [1].

For *KRAS* analysis, DNA was isolated from prepared sections of the received formalin-fixed, paraffin-embedded tissue with the DNeasy Blood & Tissue Kit (Qiagen GmbH, Germany), according to the manufacturer's protocol. The polymerase chain reaction (PCR) amplification was performed as published previously [2]. The PCR product was run on a conventional agarose gel and the genetic sequence was confirmed by routine sequencing.

Case Report

A 78-year-old man with no significant past medical history and no evidence of immunosuppression presented with a large left submandibular mass that had been increasing in size over a duration of 3 months. Clinically, there was no evidence of hypercalcemia, renal failure, peripheral lymphadenopathy or bone pain. The patient was mildly anemic with a hemoglobin level of 11.9 g/dl. Lymphocyte counts were normal. Radiological examinations (CT and MRI) showed complete replacement of the left submandibular gland by a lobulated solid mass measuring 7.7 cm in maximum dimension. No other tumorous lesions or pathologically enlarged lymph nodes were detected. After a fine needle aspiration was performed, the tumor was excised.

Subsequent full skeletal XR revealed an lytic lesion with a soap bubble appearance in the upper end of the right radial shaft. This lesion was not biopsied. Bone marrow aspirate showed a normocellular marrow with no increase in plasma cells (plasma cells were mature in appearance

and comprised 3% of total nucleated cells). No monoclonal bands were detected on serum and urine protein electrophoresis. There was no anemia or hypercalcemia and his renal function was normal. The human immunodeficiency virus (HIV) status was negative.

Results

Fine Needle Aspiration Cytology (FNAC)

Smears were highly cellular and showed abundant bare nuclei in a background of blood and proteinaceous material. The bare nuclei contained condensed chromatin in a vague cartwheel-like pattern and indistinct nucleoli, resembling lymphocytes (Fig. 1). Retrospective review of the smears showed very occasional intact plasma cells interspersed between the bare nuclei.

Gross Examination

Grossly, a pale fleshy tumor was noted to have replaced the entire salivary gland, with no definite residual salivary gland tissue identified macroscopically (Fig. 2).

Routine Histology

Microscopically, the tumor was comprised of confluent sheets of plasma cells with varying degrees of low-grade atypia. The plasma cells showed predominantly mature differentiation, and no plasmablastic features were identified. The cytoplasm was slightly to moderately basophilic with a pale paranuclear zone (Fig. 3a). No Dutcher bodies were seen. The tumor cells were seen infiltrating into surrounding skeletal muscle and salivary gland tissue

Fig. 1 FNA cytology showing abundant bare nuclei with condensed chromatin in a vague cartwheel-like pattern ($\times 600$)

Fig. 2 **a** Cut section of gross specimen showing a pale fleshy tumor. **b** Residual salivary gland tissue (arrow) is focally present (Hematoxylin and Eosin)

Fig. 3 **a** Neoplastic plasma cells infiltrate the salivary gland tissue. Note residual ducts to the right ($\times 100$). **b** Confluent sheets of plasma cells with varying degrees of low-grade atypia is present. No overt

plasmablastic features are identified ($\times 400$). **c** Strong CD38 immunohistochemical and **d** EBER in situ hybridization positivity ($\times 400$)

(Fig. 3b). No abnormal lymphoid population, monocytoid cells or lymphoepithelial lesions were identified.

Immunohistochemistry

Immunohistochemically, the tumor cells showed strong CD38 (Fig. 3c) and MUM1 expression. Lambda light chain

restriction and weak focal CD79a expression was present. EBER in situ hybridization (ISH) was also strongly positive (Fig. 3d). The tumor cells were negative for CD138, CD20, CD56, cyclin D1, CD43, CD45, AE1/3 and EMA. The proliferative index as determined by Ki67 staining ranged from 20% to 70%. CD3 and CD5 highlighted reactive T cells. Based on the clinical, morphologic and

phenotypic results, a diagnosis of EBV-positive plasmacytoma was made.

FISH Analysis

FISH analysis was negative for the t(4;14) *FGFR3/MMSET* and *IGH* translocation as well as any translocations involving the *IGH*, *IGL*, *IGK*, *CCND1*, *BCL2*, *BCL6* and *C-MYC* genes.

KRAS Mutational Analysis

No mutations of codons 12, 13 and 61 were identified.

Discussion

Plasmacytomas initially presenting as salivary gland masses are very uncommon, accounting for 0.7% of upper aerodigestive tract plasmacytomas according to Batsakis et al. [3]. Our case highlights a potential pitfall that may occur on FNAC examination of plasmacytomas particularly if they arise in unusual sites such as in the case presented herein. Although FNAC of plasma cell neoplasms generally demonstrates typical plasmacytoid cells with abundant finely granular cytoplasm and eccentric nuclei containing fine chromatin and indistinct nucleoli, occasionally numerous bare nuclei are encountered which may give rise to diagnostic difficulties [4], as illustrated in our case. The very occasional plasma cells that were present in our FNAC did not raise a suspicion of a plasma cell neoplasm initially. We speculate that an unusually high degree of fragility of the neoplastic cells in our case resulted in the loss of cytoplasm in the majority of the tumor cells.

The absence of CD138 expression (as observed in our case) is an uncommon but previously reported phenomenon in plasma cell tumors [5, 6]. Proposed mechanisms for weak/absent CD138 expression in malignant plasma cells include apoptosis (unlikely in our patient) [7] and increased shedding from the cell surface [6]. The clinical relevance of weak/absent CD138 expression is not established, but various studies suggest that plasma cell tumors with weak/absent CD138 expression exhibit more aggressive behavior [6, 8] and may be less chemosensitive [8].

Another interesting observation was the Epstein-Barr virus (EBV)-positivity. EBV positivity in various lymphoproliferative conditions is a well-recognized phenomenon. Examples include plasmablastic lymphomas (PBL), Burkitt lymphoma, AIDS-associated B-cell lymphomas, post-transplantation lymphoproliferative disorders (PTLD), lymphomatoid granulomatosis, diffuse large B-cell lymphoma (DLBCL) associated with chronic inflammation, EBV-positive DLBCL of the elderly, and extranodal

NK/T-cell lymphoma, nasal type [9]. Plasmacytomas as a group are not commonly EBV-positive, although cases of EBV-positivity in plasmacytomas are on record [10]. In one study, EBV RNA was detected in 17.4% (4/23) of EMP of the head and neck [11].

For this current case, important differential diagnostic considerations that need to be excluded in view of the histomorphological and immunohistochemical findings are extramedullary myeloma, PBL, PTLD and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Based on the criteria defined by the International Myeloma Working Group, a diagnosis of extramedullary myeloma was excluded given the lack of bone marrow involvement, absence of end-organ damage and the absence of a monoclonal band on serum protein electrophoresis [12]. As the skeletal survey showed an expansile lytic lesion in the right radial shaft for which the histological diagnosis is unavailable, we favor the diagnosis of multiple solitary plasmacytomas with both bone and extramedullary involvement.

In view of the site of occurrence and EBV-positivity, PBL is an important diagnostic consideration. This is especially true since PBL may show plasmacytic differentiation [13]. However, the complete absence of plasmablastic features in this tumor, i.e. large cells with oval nuclei, open chromatin and prominent central nucleoli argues against a diagnosis of PBL. Moreover, the absence of *C-MYC* rearrangements by FISH analysis also favors a diagnosis of plasmacytoma as *C-MYC* rearrangements are present in around 75% of EBV-positive PBL [14].

Extramedullary plasmacytoma-like PTLD has previously been reported [15] and this entity is often associated with EBV; however, the lack of a history of transplant or immunosuppression/immunodeficiency rules out a PTLD or HIV-associated lymphoma.

MALT lymphoma represents one of the commonest hematolymphoid malignancies arising in the salivary glands [16], and may exhibit prominent plasmacytic differentiation [17]. However, the absence of an abnormal lymphoid population coupled with the lack of immunohistochemical staining for CD20 render a MALT lymphoma highly unlikely.

As the genetic features of plasmacytomas in general, and EBV-positive plasmacytomas in particular, are not well studied, we attempted to characterize the molecular genetic profile of this rare entity by FISH in order to compare it with known common genetic abnormalities of multiple myeloma (MM). In our case, we did not find any translocation involving the *IgH* gene, which is the locus most frequently translocated in MM with a prevalence of around 55–70% [18, 19]. The absence of a breakapart involving the *IGH* gene in our case is in line with the study by Bink et al. [20], which showed in their cohort of 38

EMPs that only a third contained *IGH* breaks. The absence of translocations involving the *CCND1*, *BCL6* and *C-MYC* genes in our case are also consistent with their study which showed the absence of t(8;14) and t(11;14) translocations as well as breaks involving the *BCL6* gene. In their study, the t(4;14) *IGH/FGFR3* occurred in 16% of EMPs; this was absent in our case. Although no data are available regarding the prevalence of *IGL* and *IGK* breaks in plasmacytomas, the reported incidence in one study of MM was 18% [21]. Overall, the genetic profile of our [18, 19] case is more in keeping with plasmacytoma than MM [20].

The presence of *KRAS* mutations has been previously reported in MM [22–24] and correlate with a poorer overall survival and progression-free survival [24]. The prevalence of *KRAS* mutations in plasmacytomas is not well characterized. As one study had previously reported the presence of *RAS* mutations only in extramedullary plasma cells and not in intramedullary plasma cells in patients with extramedullary plasma cell tumors [22], we were interested in examining our case for the presence of *KRAS* mutations. Our analysis did not reveal any mutations in codons 12, 13 and 61.

As regards their natural history, plasmacytomas are generally associated with a more favorable prognosis compared to MM. In one recent study, 5-year survival rates for plasmacytomas as a group ranged from approximately 40 to 70% for patients aged 60 years and above, in contrast to a 5-year survival rate of around 26% for MM patients of similar age [25]. Plasmacytomas may occasionally progress to myeloma as well [26]. Also, EBV-positivity has been associated with a more aggressive clinical course in other hematolymphoid malignancies [27, 28]; subsequent follow-up information of our patient will be required to ascertain the biological behavior of this tumor.

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