The Korean Journal of Pathology 2014; 48: 335-338
http://dx.doi.org/10.4132/KoreanJPathol.2014.48.4.335

■ BRIEF CASE REPORT ■

Usefulness of Nuclear Protein in Testis (NUT) Immunohistochemistry in the Cytodiagnosis of NUT Midline Carcinoma: A Brief Case Report

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Nuclear protein in testis (NUT) midline carcinomas are uncommon, recently described, fatal neoplasm that are characterized by rearrangement of the *NUT* gene on 15q14.[1] Since the first case of NUT midline carcinoma was reported as an undifferentiated carcinoma with a t(15;19) translocation in 1991,[2] more than 50 cases have been reported.[3] Because of the unique chromosomal translocation and predilection for occurrence in midline structures, they have been termed NUT midline carcinomas. However, tumors outside the midline have been reported, including the salivary gland, bladder, and other sites.[1,3-5] We encountered a case of NUT midline carcinoma confirmed by NUT specific immunohistochemistry (IHC) using cytologic and histologic specimens. Herein, we present the first case report on immunocytologic features of NUT midline carcinoma arising in the parotid gland.

CASE REPORT

A 12-year-old boy without a significant past medical history presented with a parotid mass. The neck magnetic resonance imaging (MRI) revealed a 2.4-cm-sized mass with irregular margins in the deep subcutaneous fat layer of the right cheek (Fig. 1). Several other masses with similar characteristic signal changes as the right cheek mass were identified in the deep fat layer in the right mandibular angle and submandibular area.

A fine needle aspiration was performed on the right cheek mass. The aspirated smear had a necrotic background and showed both syncytial tissue fragments and scattered single cells (Fig. 2). The smear showed tiny necrotic foci. The tumor cells were relatively small (nucleus about 9 μm, cytoplasm about 20 μm in the longest diameter) and had pale cytoplasm and indistinct cell borders. The tumor nuclei were ovoid with small nucleoli and the chromatin was evenly dispersed and hyperchromatic. The nuclear membranes had slight irregularity with occasional grooves. Most single cells had naked nuclei and occasional mitotic figures were identified. Squamous or glandular differentiation was not evident. A right total parotidectomy with neck dissection was performed for tumor removal and pathologic confirmation of the tumor subtype.

Microscopically, it revealed numerous tumor islands forming sheets or nests within the desmoplastic stroma (Fig. 3). The tumor cells had similar cytologic characteristics to those of the aspirated smear (Fig. 3). Metastasis to the cervical lymph nodes as well as perineural and lymphatic invasion was found. As the tumor cells did not demonstrate any differentiation, diverse immunohistochemical stains including pancytokeratin (Leica Microsystem, Novocastra, Newcastle upon Tyne, UK), vimentin (Dako, Carpinteria, CA, USA), p63 (Biogenex, San Ramon, CA, USA), S-100 (Dako), CD34 (Leica Microsystem), synaptophysin (Leica Microsystem), chromogranin A (Leica Microsystem), CD56 (Leica Microsystem), smooth muscle actin (Dako), desmin (Dako), Wilms tumor-1 (Leica Microsystem), and CD99 (Dako) were performed. The tumor cells expressed only pancytokeratin and p63 (Fig. 3).

At first glance, the most possible diagnosis of this case was poorly differentiated squamous cell carcinoma based on the immunohistochemical staining results. However, we hesitated to report squamous cell carcinoma owing to the patient's young

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Received: January 22, 2014 **Revised:** April 9, 2014
Accepted: April 11, 2014

pISSN 1738-1843
eISSN 2092-8920

age, and lack of smoking history or squamous differentiation on light microscopic examination. Finally, we performed the NUT (1:100, C52B1, Cell Signaling Technology, Danvers, MA, USA) immunostain using tissue section as well as cell block section from the aspirate. The tumor nuclei showed diffuse strong immunoreactivity to NUT and we were able to diagnose NUT midline carcinoma (Figs. 2, 3).

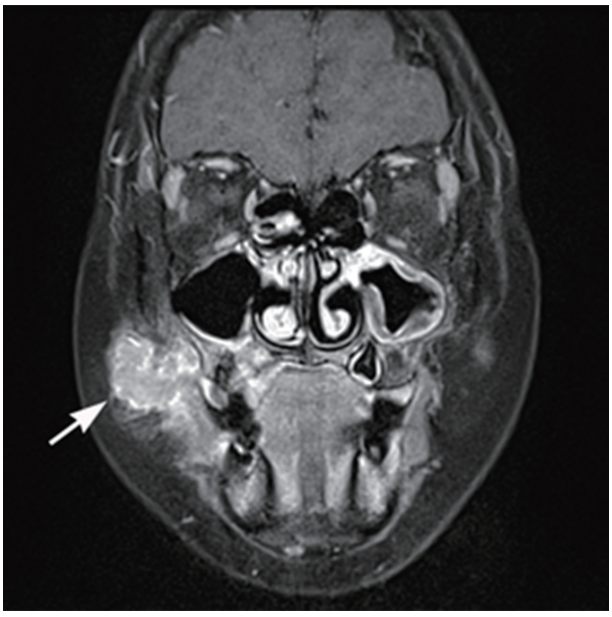

Fig. 1. Radiologic findings. Neck magnetic resonance imaging reveals an infiltrative mass (arrow) with strong gadolinium enhancement, located in the deep fat layer of the right cheek.

Despite intensive postoperative chemo-radiation therapy, two recurrent events occurred within 1 year after the initial operation. The patient experienced disease progression and expired 2 years after the first presentation.

DISCUSSION

NUT midline carcinoma is a rare and aggressive subset of squamous cell carcinoma, genetically defined by chromosomal translocation involving the *NUT* gene.[1] In about two thirds of NUT midline carcinomas, *NUT* is fused to *BRD4* [t(15;19)] and in the remainder of cases, it is either fused to *BRD3* [t(9;15)] or to unknown partner (NUT-variant).[4] This cancer affects primarily the mediastinum, head and neck, but it arises in many other organs, such as the urinary bladder, iliac bone, pancreas, parotid gland, and lung.[3-5] It occurs in people of any ages (range, 0 to 78 years) or gender. NUT midline carcinoma is refractory to

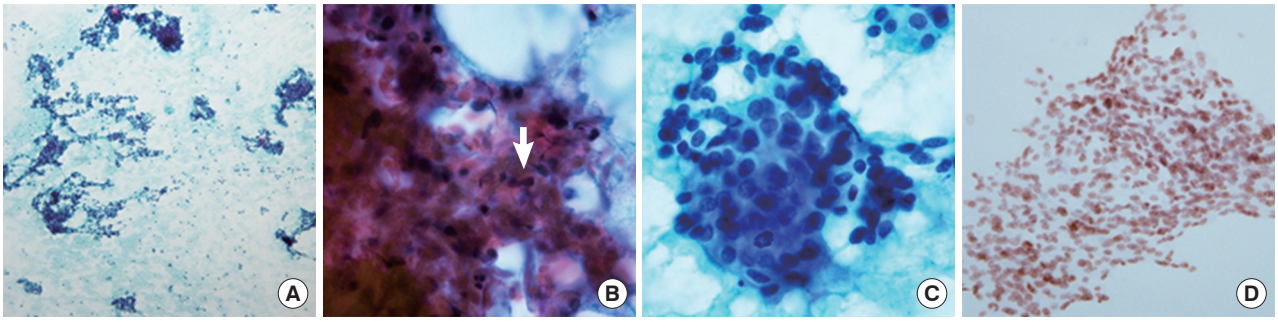

Fig. 2. Cytologic findings. (A) The smear shows many cohesive clusters with single scattered cells. (B) Tumor cell necrosis is found. (C) The tumor cells are relatively small and uniform with irregular ovoid nuclei, small nucleoli, and occasional mitosis. (D) NUT immunohistochemistry (C52) using the aspirated smear slide reveals diffuse nuclear immunoreactivity.

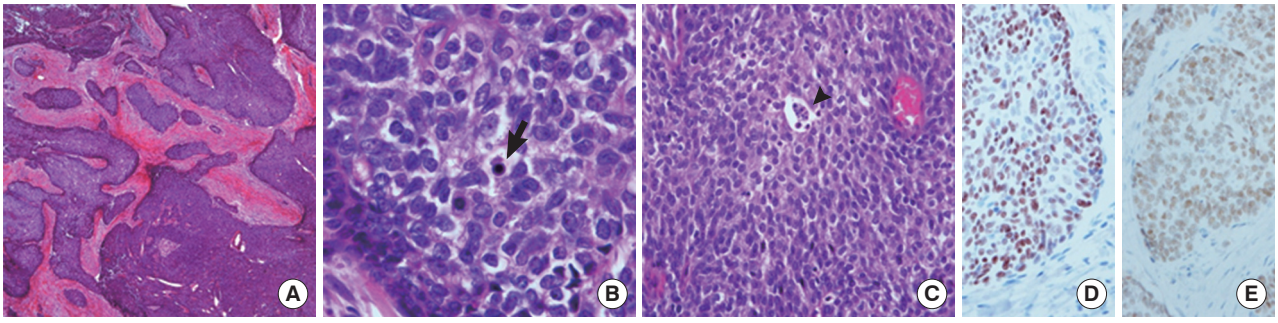

Fig. 3. Histopathologic findings. (A) The tumor is composed of sheets of poorly differentiated cells with desmoplastic stroma. (B, C) The tumor cells have relatively small and irregular ovoid nuclei with small nucleoli. Individual cell necrosis (arrow), frequent mitosis, and tiny necrotic foci (arrowhead) are present. Immunohistochemical stains reveal diffuse nuclear immunoreactivity to p63 (D) and the NUT specific antibody (E).

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conventional chemotherapy and radiotherapy. It has a fatal clinical course with median survival of 6.7 months.[6] Two therapeutic agents targeting the *BRD4-NUT* oncogene (bromodomain inhibitors and histone deacetylase inhibitors) have emerged and are in phase I clinical trials.[4] Therefore, an early and proper diagnosis of NUT midline carcinoma would become essential.

Unfortunately, the histopathology of NUT midline carcinomas is not diagnostic. They are composed of sheets or nests of undifferentiated tumor cells with varying degrees of squamous differentiation.[4,7] Large areas of coagulative necrosis and extensive desmoplastic stroma may be present.[7] Their morphology overlaps with that of many other poorly differentiated malignant neoplasm and NUT midline carcinoma is frequently misdiagnosed as squamous cell carcinoma, poorly differentiated carcinoma, sinonasal undifferentiated carcinoma, Ewing sarcoma, leukemia, thymic carcinoma (if in the thorax), or neuroblastoma.[7,8] However, there are a few morphologic features which could suggest NUT midline carcinoma such as relatively monotonous and round tumor cells and foci of abrupt squamous differentiation in about 50% of cases.[3,4,7]

Bellizzi *et al*.[9] and Zhu *et al*.[10] described the cytologic features of NUT translocated carcinoma. The cytologic characteristics was a highly cellular, predominantly discohesive pattern of relatively small tumor cells with a round nucleus, scant cytoplasm, irregular nuclear contours, variably prominent nucleoli, and identifiable mitotic figures. Overt keratinization, which may be typical in surgical specimens, was not identified, while occasional cells with a denser cytoplasm were noted in one case. The original cytologic diagnoses were "consistent with sinonasal undifferentiated carcinoma" and "consistent with metastatic carcinoma." Distinction of NUT midline carcinoma from other undifferentiated carcinomas without any ancillary tests is nearly impossible in cytology specimens. However, despite the absence of pathognomic cytologic or histologic features, it is critical to know this entity and to consider confirmative tests because NUT midline carcinoma has a very aggressive clinical course and might be managed by other types of chemotherapeutic regimens.

For a confirmative diagnosis of NUT midline carcinoma, karyotyping, fluorescence *in situ* hybridization (FISH), or *NUT* mRNA by reverse transcription polymerase chain reaction can be used to detect the *NUT* gene rearrangement.[4] However, these tests are not widely available and have not been commercialized. Recently, both monoclonal[8] and polyclonal[7] NUT specific antibodies were developed for IHC. Haack *et al*.[8] assessed the accuracy of monoclonal NUT (C52) antibody to detect NUT rearranged carcinoma confirmed by FISH. They demonstrated that NUT IHC had a sensitivity of 87% and a specificity of 100% when diagnosing NUT midline carcinoma among nongerm cell tumors. Generally, germ cells in the testis and oocytes are stained with NUT antibody. Germ cell tumors such as dysgerminoma, seminoma, and embryonal carcinoma were reported to show focal (<5%), weak nuclear staining with C52, presumably due to the normal expression of NUT in tumor cells, whereas NUT midline carcinomas showed diffuse (>50%), speckled nuclear staining.[8] Thus, IHC using the NUT specific antibody can be used as an alternative diagnostic test if the tumor cells show diffuse immunoreactivity to the NUT specific antibody.

Almost all NUT midline carcinomas show immunoreactivity to p63,[5,7,9] reflecting the squamous nature of this tumor. However, it is very unique in that it is characterized by a single chromosomal translocation like leukemia/lymphoma, in contrast to complex karyotypes and aneuploidy in typical squamous cell carcinomas. In addition, none of the smoking-related squamous cell carcinoma of the head and neck showed NUT immunostaining or gene rearrangement.[8] This suggests that NUT midline carcinoma might arise through a different pathogenesis. The *NUT* oncogene may represent a short-cut to squamous cancer, bypassing multiple processes of environmentally-acquired mutations and genetic instability in conventional squamous cell carcinoma.[3,4]

In this report, we presented a case of NUT midline carcinoma, which developed in the parotid gland of an adolescent boy. NUT midline carcinoma is a recently described, uncommon disease with a fatal outcome and is underdiagnosed due to nonspecific morphologic features. In cases with poorly differentiated malignancy of the head and neck, it is important to be aware of this entity and to perform NUT immunostaining in a cytology specimen for proper diagnosis and patient management.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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