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

Heae Surung Park · Yoon Sung Bae · Sun Och Yoon · Beom Jin Lim · Hyun Jun Hong1 · Jae Y Ro2 · Soon Won Hong

Departments of Pathology and 1Otolaryngology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; 2Department of Pathology, The Methodist Hospital and Weill Medical College of Cornell University, Houston, TX, USA

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.4-5 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 Microsystems, 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 (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 age, presentation, and imaging findings.
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).

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
conventional chemotherapy and radiotherapy. It has a fatal clinical course with median survival of 6.7 months. Two therapeutic agents targeting the BRD4-NUT oncogene (bromodomain inhibitors and histone deacetylase inhibitors) have emerged and are in phase I clinical trials. 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. Large areas of coagulative necrosis and extensive desmoplastic stroma may be present. 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. 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.

Bellizzi et al. and Zhu et al. 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 metastic 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 pathognomonic 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. However, these tests are not widely available and have not been commercialized. Recently, both monoclonal and polyclonal NUT specific antibodies were developed for IHC. Haack et al. 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. 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, 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. 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.

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.

REFERENCES

1. French CA, Kutok JL, Faquin WC, et al. Midline carcinoma of children and young adults with NUT rearrangement. J Clin Oncol 2004;22:4135-9.
2. Kubonishi I, Takehara N, Iwata J, et al. Novel t(15;19)(q15;p13) chromosome abnormality in a thymic carcinoma. Cancer Res 1991;51:3327-8.
3. French CA. The importance of diagnosing NUT midline carcinoma. Head Neck Pathol 2013; 7: 11-6.
4. French CA. Pathogenesis of NUT midline carcinoma. Annu Rev Pathol 2012; 7: 247-65.
5. den Bakker MA, Beverloo BH, van den Heuvel-Eibrink MM, et al. NUT midline carcinoma of the parotid gland with mesenchymal differentiation. Am J Surg Pathol 2009; 33: 1253-8.
6. Bauer DE, Mitchell CM, Strait KM, et al. Clinicopathologic features and long-term outcomes of NUT midline carcinoma. Clin Cancer Res 2012; 18: 5773-9.
7. Stelow EB, Bellizzi AM, Taneja K, et al. NUT rearrangement in undifferentiated carcinomas of the upper aerodigestive tract. Am J Surg Pathol 2008; 32: 828-34.
8. Haack H, Johnson LA, Fry CJ, et al. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. Am J Surg Pathol 2009; 33: 984-91.
9. Bellizzi AM, Bruzzi C, French CA, Stelow EB. The cytologic features of NUT midline carcinoma. Cancer 2009; 117: 508-15.
10. Zhu B, Laskin W, Chen Y, et al. NUT midline carcinoma: a neoplasm with diagnostic challenges in cytology. Cytopathology 2011; 22: 414-7.