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NUT Midline Carcinomas of the Sinonasal Tract

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Abstract

NUT midline carcinoma (NMC) is a highly lethal tumor defined by translocations involving the *NUT* gene on chromosome 15q14. NMC involves midline structures including the sinonasal tract, but its overall incidence at this midline site and its full morphologic profile are largely unknown because sinonasal tumors are not routinely tested for the *NUT* gene translocation. The recent availability of an immunohistochemical probe for the NUT protein now permits a more complete characterization of sinonasal NMCs. The archival files of The Johns Hopkins Hospital Surgical Pathology were searched for all cases of primary sinonasal carcinomas diagnosed from 1995 to 2011. Tissue microarrays were constructed, and NUT immunohistochemical analysis was performed. All NUT-positive cases underwent a more detailed microscopic and immunohistochemical analysis. Among 151 primary sinonasal carcinomas, only 3 (2%) were NUT positive. NUT positivity was detected in 2 of 13 (15%) carcinomas diagnosed as sinonasal undifferentiated carcinoma and in 1 of 87 (1%) carcinomas diagnosed as squamous cell carcinoma. All occurred in men (26, 33, and 48 y of age). The NMCs grew as nests and sheets of cells with a high mitotic rate and extensive necrosis. Two were entirely undifferentiated, and 1 tumor showed abrupt areas of squamous differentiation. Each case had areas of cell spindling, and 2 were heavily infiltrated by neutrophils. Immunohistochemical staining was observed for cytokeratins (3 of 3), epithelial membrane antigen (3 of 3), p63 (2 of 3), CD34 (1 of 3), and synaptophysin (1 of 3). All patients died of the disease (survival time range, 8 to 16mo; mean, 12mo) despite combined surgery and chemoradiation. NMC represents a rare form of primary sinonasal carcinoma, but its incidence is significantly increased in those carcinomas that exhibit an undifferentiated component. Indiscriminant analysis for evidence of the NUT translocation is unwarranted. Instead, NUT analysis can be restricted to those carcinomas that demonstrate undifferentiated areas. The availability of an immunohistochemical probe has greatly facilitated this analysis and is helping to define the full demographic, morphologic, and immunohistochemical spectrum of sinonasal NMC.

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Keywords

NUT midline carcinoma; sinonasal tract; sinonasal undifferentiated carcinoma; t(15;19) translocation; *BRD4-NUT*

BACKGROUND

NUT midline carcinoma (NMC) is a recently described malignancy that is defined by balanced translocations involving the *NUT* (nuclear protein in testis) gene on chromosome 15q14.^{6,7} In approximately two thirds of cases, the translocation occurs with the *BRD4* (bromodomain-containing protein 4) gene on 19p13.1, leading to a *BRD4-NUT* fusion oncogene.⁷ The remaining cases have a different translocation partner.⁵

NMC is an aggressive and almost uniformly lethal tumor with a propensity for early hematogenous spread. The mean patient survival time is only 9 months.⁴ Although these tumors may not respond to standard therapeutic protocols for head and neck squamous cell carcinoma, 1 patient was cured of an NMC when treated with an Ewing sarcoma protocol.¹⁰ The presence of a consistent chromosomal rearrangement may provide a specific target for biological therapeutic agents. Indeed, preliminary studies using histone deacetylase inhibitors and BET inhibitors have shown promising results both in vitro and in vivo,^{3,5,11} and clinical trials using these agents are forthcoming.⁵ Clearly, the recognition of NMC is very important from both a prognostic and therapeutic perspective.

Despite the importance of recognizing NMCs, they are almost certainly underdiagnosed. In the head and neck, the sinonasal tract is considered a preferential site, but documented sinonasal NMCs are limited to 7 reported cases.^{2,6,9,14} There are a number of factors that may contribute to the underdiagnosis of sinonasal NMCs. First, NMC is a recently described tumor entity that may be unfamiliar to most pathologists. Second, the morphologic and immunohistochemical features of NMCs overlap with other poorly differentiated carcinomas of the sinonasal tract such that a definitive diagnosis of NMC based solely on histology and immunohistochemistry is not considered possible.¹³ Third, many diagnostic laboratories do not have easy access to the molecular genetic resources needed to detect *NUT* gene rearrangements. In the absence of such resources, poorly differentiated carcinomas of the sinonasal tract are not routinely tested for the presence of the diagnostic chromosomal rearrangements. As a result, the true incidence of sinonasal NMCs is not known, and the frequency with which these tumors are misdiagnosed as some other tumor type is undefined.

The recent development of a highly sensitive and specific monoclonal antibody for the NUT protein has greatly simplified the recognition of NMC. In a study that evaluated a panel of over 1000 tissue types including a diverse spectrum of carcinomas, immunohistochemical staining for the NUT protein was found to have a negative predictive value of 99% and a positive predictive value of 100%.⁸ The availability of a highly predictive immunohistochemical assay has paved the way for largescale testing of head and neck carcinomas in a way that can establish the incidence of NMCs at specific sites and define the limits of its demographic, microscopic, and immunohistochemical profile.

METHODS

Cases

The Johns Hopkins Hospital Surgical Pathology archives were searched for all cases of primary sinonasal carcinoma diagnosed from 1995 to 2011. Cases included biopsies and resection specimens. Carcinomas arising from the nasopharynx, skin, or odontogenic apparatus and involving the sinonasal tract secondarily were not included. The slides were reviewed, and a formalin-fixed and paraffin-embedded tissue block was selected. Cases later found to be NMC were rereviewed, and their histologic features were further detailed.

Tissue Microarray

Tissue microarrays were constructed using the sinonasal carcinoma tissue blocks. The microarrays included 151 cases of sinonasal carcinoma from patients ranging from 26 to 93 years. The tumors included 87 squamous cell carcinomas and variants, 34 salivary gland carcinomas [including 27 adenoid cystic carcinomas, 3 myoepithelial carcinomas, 2 mucoepidermoid carcinomas, and 1 salivary adenocarcinoma not otherwise specified (NOS)], 13 sinonasal undifferentiated carcinomas (SNUCs), 7 nonsalivary adenocarcinomas (including 4 low-grade nonintestinal adenocarcinomas, 2 high-grade nonintestinal adenocarcinomas, and 1 intestinal adenocarcinoma), 5 neuroendocrine carcinomas, and 5 carcinomas NOS. Two core samples were taken from each block to address the issue of tumor heterogeneity.

Immunohistochemistry

Five-micrometer-thick sections of formalin-fixed and paraffin-embedded tissues were deparaffinized and subjected to antigen retrieval using EDTA buffer (pH 9.0). NUT expression was determined using a monoclonal rabbit antibody against NUT (clone C52B1; Cell Signaling Technologies Inc., Danvers, MA; 1:50 dilution) using a biotin-free polymer detection kit (Leica Microsystems, Bannockburn, IL). Formalin-fixed, paraffin-embedded tissue blocks containing non-neoplastic testis tissue were used as positive controls. As previously described,⁸ only strong, diffuse (> 50% of cells) nuclear staining was regarded as positive. Cases found to be NMC were additionally studied with p63 (clone 4A4; Cell Marque, Rocklin, CA; prediluted by manufacturer), synaptophysin (clone 27G12; Leica Microsystems; prediluted), chromogranin (clone LK2H10; Ventana, Tuscon, AZ; prediluted), AE1/AE3 (clone AE1/AE3/PCK26; Ventana; prediluted), CD34 (clone QBEnd/10; Ventana; prediluted), and S100 (clone 4C4.9; Ventana; prediluted).

RESULTS

Results of NUT immunohistochemistry have been summarized in Table 1. Among 151 primary sinonasal carcinomas, 3 (2%) were immunoreactive for NUT. The positive cases included 2 of 13 (15%) cases originally diagnosed as SNUC and 1 of 87 (1%) cases previously diagnosed as squamous cell carcinoma. All salivary gland carcinomas, nonsalivary adenocarcinomas, neuroendocrine carcinomas, and carcinomas NOS were negative for NUT. The testis control tissue was consistently immunoreactive for NUT in a strong and diffuse manner.

All NMCs occurred in men. These patients were 26, 33, and 48 years of age (mean, 36 y). Two patients were white, and 1 was African American. Two patients had never smoked, and 1 was a former smoker (information on pack-years was not available). The presenting signs and symptoms were nasal mass and pain in 1 patient, proptosis in another patient, and toothache in the third patient. All 3 NMCs were centered in a paranasal sinus (2 maxillary and 1 ethmoid) with extensive local invasion at the time of presentation. Each patient was treated with surgery, along with adjuvant systemic chemotherapy and external beam radiation. The chemotherapy regimens consisted of cisplatin and 1 other agent (Adriamycin, 5-fluorouracil, and Taxol, respectively). One patient received 3000 cGy in 12 fractions at 250 cGy/fraction to his spine, but the details of the radiotherapy for the other 2 patients were not available. Although there were no metastases at the time of presentation, all 3 developed metastatic disease (to bone in 3 and to lymph nodes in 2) and died. The survival times were 8, 11, and 16 months (mean, 12mo).

Histologically, the 2 cases originally regarded as SNUC showed no evidence of differentiation by routine hematoxylin and eosin staining. The NMCs grew as sheets, nests, and cords of cells (Fig. 1A). They were histologically high grade with abundant mitoses, apoptotic bodies, and zones of tumor necrosis (Fig. 1B). The NMC cells had a moderate amount of eosinophilic to amphophilic cytoplasm, round to oval nuclei with 1 or more prominent nucleoli, and chromatin ranging from finely granular to vesicular (Fig. 1C). Despite the high-grade features, the tumor nuclei lacked the pleomorphism that is characteristic of many poorly differentiated carcinomas. The third case, originally diagnosed as squamous cell carcinoma, predominantly consisted of undifferentiated cells but with areas of overt squamous differentiation. For the most part, the transition to squamous cells was abrupt, with scattered foci of keratinized clear cells immediately adjacent to the undifferentiated cells (Fig. 1D). Focally, the squamous differentiation was more conventional in its appearance, with areas of abundant pink cytoplasm, extracellular keratin formation, and the presence of cystic degeneration. Each case was widely infiltrative, showing invasion of bone and lymphovascular spaces. Perineural invasion was seen in 1 case. Although the NMC cells were mostly epithelioid, all 3 cases had focal areas of cell spindling (Fig. 2B). No glands, mucin, or rosettes were seen in any case. Two of the NMCs had very prominent infiltration by neutrophils, including scattered microabscesses (Fig. 2A). In each NMC, crush artifact was abundant, and in 2 cases this artifact contributed to a nondiagnostic frozen section diagnosis of “crushed atypical cells.” In each NMC, the overlying surface consisted of unremarkable respiratory epithelium with occasional foci of squamous metaplasia. There was no histologic evidence of premalignant changes in any case.

The immunohistochemical profiles of the NMCs have been summarized in Table 2. Each NMC was positive for the cytokeratin cocktail AE1/AE3. The cytokeratin expression was diffuse but of variable intensity. Epithelial membrane antigen was also positive in each case, although the expression was only focal in 2 cases. p63 was strongly and diffusely positive in 2 NMCs but was entirely negative in 1 case. One of the NMCs was diffusely CD34 positive, and the same case also showed focal positivity for synaptophysin, chromogranin (Fig. 3A), and S100 (Fig. 3B). Each case showed strong, diffuse nuclear NUT expression (Fig. 3C),

except in areas of overt squamous differentiation where staining was negative or weak (Fig. 3D).

DISCUSSION

By testing a large number of unselected sinonasal carcinomas using NUT immunohistochemistry, we found that NMC represents 2% of all carcinomas arising in the sinonasal tract. Although NMC was rare overall, it did constitute a significant number of tumors previously diagnosed as SNUC (2 of 13, 15%) or as nasal carcinomas in patients younger than 50 years (3 of 45, 7%). In fact, most patients younger than 50 years diagnosed with SNUC (3 of 4, 75%) were found to actually have NMCs. Clinically, the NMCs from our series conformed to what has been reported about this tumor. NMC has been documented in patients of all ages, from newborns¹² to those aged 78 years,¹⁴ but has been seen primarily in children and young adults.⁵ Our series did not have any pediatric sinonasal cancers (the youngest patient was 26 years old), but all 3 NMCs occurred in patients younger than 50 years. All of the NMC patients in our series died within 18 months of diagnosis, consistent with the devastating clinical course reported in most cases.

In an analysis that included all of the major types of carcinomas that arise in the sinonasal cavity, the presence of NUT staining was restricted to those carcinomas with undifferentiated areas. As previously noted, these undifferentiated areas were characterized by sheets of uniform cells with high-grade features. When squamous differentiation was observed, it was often present as zones of abrupt keratinization.^{4,5,14} We also observed morphologic features that have not been as emphasized in the literature. A prominent neutrophilic infiltrate was noted in 2 of 3 cases, and foci of cell spindling were observed in all 3 cases. More experience will be needed to determine how common these histologic features are in sinonasal NMCs, but an appreciation for the full morphologic spectrum of NMC may help formulate the differential diagnosis and guide the use of additional ancillary diagnostic tests. It is interesting to note that the 11 SNUCs that were NUT negative (ie, not NMCs) were not neutrophil rich, although slight cell streaming was seen occasionally (4/11 cases).

The immunohistochemical findings of the 3 NMCs were largely in line with what is known about this tumor, and they were not very specific. For example, strong and diffuse p63 immunoreactivity is typical of NMC¹³ but is also seen in many other tumor types, including almost all squamous cell carcinomas. Strong p63 expression would favor NMC over SNUC,¹ but NMC can also be only focally p63 positive¹⁴ or, as we observed in 1 case, entirely negative. As another example, expression of neuroendocrine markers in an undifferentiated nasal tumor would raise concern for a high-grade olfactory neuroblastoma. However, as illustrated by 1 case in this series, synaptophysin and chromogranin can be focally positive in NMC.

Ultimately, the diagnosis of NMC rests entirely on documenting evidence of an *NUT* gene rearrangement, and the recent availability of a highly accurate NUT immunohistochemical stain has greatly assisted in this testing. In this study, interpretation of NUT immunoreactivity in the tumors was straightforward, and non-neoplastic testis served as an

excellent control tissue. Given the overall rarity and relatively limited phenotype of NMCs, however, routinely testing all sinonasal cancers with the NUT antibody is not necessary. Rather, it is reasonable to include the NUT immunostain as a part of the diagnostic workup of any sinonasal carcinoma with an undifferentiated component. This group includes not only tumors meeting diagnostic criteria for SNUC but also very poorly differentiated carcinomas exhibiting squamous differentiation.

In summary, NMC is a recently described tumor that should be diagnosed accurately for prognostic and therapeutic reasons. Its clinical, morphologic, and immunohistochemical features are not very specific; therefore, testing specifically for *NUT* gene alterations is necessary to conclusively distinguish NMC from other sinonasal tumors with overlapping attributes. NMC is rare overall in the sinonasal tract. However, its incidence is significant among carcinomas that contain undifferentiated areas, particularly those arising in patients younger than 50 years. As a result, *NUT* analysis may be limited to those sinonasal carcinomas with an undifferentiated component. NUT immunohistochemistry has greatly simplified this testing, and its widespread availability will increase the recognition of sinonasal NMC.

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FIGURE 1.

Histology of sinonasal NMCs. A, The tumors grew as sheets and nests in the sinonasal submucosa, without involving the overlying epithelium (hematoxylin and eosin). B, The tumors were high grade, exhibiting zones of tumor necrosis (hematoxylin and eosin). C, The tumor cells were loosely cohesive with eosinophilic to amphophilic cytoplasm and uniform round to oval nuclei (hematoxylin and eosin). D, One case exhibited squamous differentiation, which usually took the form of an abrupt transition to clear cells with abundant cytoplasmic keratin (hematoxylin and eosin).

FIGURE 2.

Histology of sinonasal NMCs. A, Two of the carcinomas were heavily infiltrated by neutrophils that collected in areas as microabscesses (hematoxylin and eosin). B, All 3 cases exhibited foci of cell spindling (hematoxylin and eosin).

FIGURE 3.

Immunohistochemistry in sinonasal NMCs. A, One of the sinonasal NMCs focally expressed neuroendocrine markers (chromogranin immunohistochemistry), as well as (B) S100 protein (S100 immunohistochemistry). C, Each sinonasal NMC was strongly and diffusely immunoreactive with NUT, a protein expressed only in NMC and germ cells (NUT immunohistochemistry). The pattern of expression was similar to that seen in the spermatids of non-neoplastic testis (inset; NUT immunohistochemistry). D, However, NUT expression was weak or even negative in the areas of overt squamous differentiation (NUT immunohistochemistry).

TABLE 1**NUT Immunohistochemistry in Sinonasal Carcinomas**

Original Histologic Diagnosis	Number Tested	NUT Positive (%)
Squamous cell carcinoma and variants	87	1 (1)
Salivary-type carcinoma	34	0
SNUC	13	2 (15)
Nonsalivary adenocarcinoma	7	0
Neuroendocrine carcinoma	5	0
Carcinoma NOS	5	0
Total	151	3 (2)

TABLE 2

Immunohistochemical Profile of NMCs

Cases	Original Diagnosis	Immunohistochemical Staining							
		NUT	AE1/AE3	p63	EMA	CD34	SYN	CHR	S100
1	SNUC	+	+	-	+	-	-	-	-
2	SNUC	+	+	+	F	+	F	F	F
3	SqCC	+	+	+	F	-	-	-	-

CHR indicates chromogranin; EMA, epithelial membrane antigen; F, focal immunorexpression (ie, <5% of tumor cells); SqCC, poorly differentiated squamous cell carcinoma; SYN, synaptophysin.