ORIGINAL REPORT

Midline Carcinoma of Children and Young Adults With *NUT* Rearrangement

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ABSTRACT

Purpose

A balanced chromosomal translocation, t(15;19), resulting in the *BRD4-NUT* oncogene, has been identified in a lethal carcinoma of young people, a disease described primarily in case reports. We sought to amass a more definitive series of tumors with *NUT* and/or *BRD4* gene rearrangements and to determine distinct clinicopathologic features.

Patients and Methods

Carcinomas (N = 98) in young individuals (median age, 32.5 years) were screened for *NUT* and *BRD4* rearrangements using dual-color fluorescence in situ hybridization. Four published carcinomas with *BRD4* and *NUT* rearrangements were also evaluated. Immunophenotypic analyses were performed.

Results

Eleven tumors had *NUT* gene rearrangements, including eight with *BRD4-NUT* fusions and three with novel rearrangements, which were designated as *NUT* variant. All *NUT*-rearranged carcinomas (NRCs) arose from midline epithelial structures, including the first example arising below the diaphragm. Patients were young (median age, 17.6 years). Squamous differentiation (seen in 82% of NRCs) was particularly striking in *NUT*-variant cases. In this first description of *NUT*-variant carcinomas, the average survival (96 weeks, n = 3) was longer than for *BRD4-NUT* carcinomas (28 weeks, n = 8). Strong CD34 expression was found in six of 11 NRCs but in zero of 45 *NUT* wild-type carcinomas.

Conclusion

NRCs arise from midline structures in young people, and NRCs with *BRD4-NUT* are highly lethal, despite intensive therapies. *NUT*-variant carcinomas might have a less fulminant clinical course than those with *BRD4-NUT* fusions. CD34 expression is characteristic in NRCs and, therefore, holds promise as a diagnostic test for this distinctive clinicopathologic entity.

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INTRODUCTION

Rare reports have described a carcinoma with translocation t(15;19)(q13, p13.1), which occurs in young people and seems to be associated with a highly lethal clinical course.[1-7] Interest in this cancer derives from its unique chromosomal translocation, which is the sole identifier of the disease. The simplicity of the karyotype in these carcinomas is striking because most carcinomas have highly complex karyotypes and lack diagnostic balanced translocations.[8] In this respect, the cytogenetic profile in t(15;19) carcinoma is more akin to those in many lymphomas and sarcomas. We recently reported that the t(15;19) results in a novel fusion oncogene, *BRD4-NUT*.[9] The limited literature on t(15;19) carcinoma suggests that this entity arises from thymic or respiratory epithelium and is invariably lethal and unresponsive to aggressive chemoradiotherapy.[1-7] To better define the

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clinicopathologic features of this disease, we sought to characterize a larger series comprised of both new cases identified by screening 98 carcinomas with a dual-color fluorescence in situ hybridization (FISH) assay for *NUT* and *BRD4* rearrangements, and cases with known t(15;19).[1,5-7]

PATIENTS AND METHODS

Patients

Hematoxylin and eosin–stained or unstained, 4-μm, formalin-fixed, paraffin-embedded sections of malignant tumors were collected based on one or more of the following criteria: epithelial differentiation, patient age less than 40 years, poorly differentiated histomorphology, rapid clinical progression, or cytogenetic evidence of chromosome 15q or 19p rearrangement. Histomorphology was reviewed in all cases. Mitotic counts (per 10 high-power fields) were performed for all *NUT*-rearranged carcinomas (NRCs). Hospitals from which tissue was obtained included Brigham and Women's Hospital, Massachusetts General Hospital, and Children's Hospital (Boston, MA), University of Maryland School of Medicine, The Johns Hopkins University School of Medicine (Baltimore, MD), Memorial Sloan-Kettering Cancer Center (New York, NY), Aghi Sofia Children's Hospital/ Athens University (Athens, Greece), and Kochi Medical School (Kochi, Japan). Clinical history and follow-up for patients harboring *NUT* rearrangements were obtained from hospital computer records or from the patient's oncologist. These studies were performed in accordance with Institutional Review Board protocol 2000-P-001990/3 of the Brigham and Women's Hospital.

FISH

Dual-color FISH assays evaluating chromosome 19p13.1 *BRD4* and 15q13 *NUT* break points were performed on formalin-fixed, paraffin-embedded, unstained, 4-μm sections as previously described.[6] Probes used for the 19p13.1 break point included telomeric bacterial artificial chromosome (BAC) clone 87m17 (green) and centromeric yeast artificial chromosome (YAC) clone 766e7 (red). Probes used for the 15q13 break point, flanking a 181-kb region, included telomeric BAC clones 1H8 and 64o3 (green) and centromeric clones 412e10 and 3d4 (red). For the first group of 40 patients, both *BRD4* and *NUT* rearrangements were evaluated by FISH. In the second group (n = 61), *NUT* rearrangements were evaluated first, followed by *BRD4* in *NUT*-rearranged patients. Patients with more than 80% hybridization efficiency in four areas (200 cells/area) of the tissue section were regarded as interpretable.

Immunohistochemistry

Immunophenotypic analysis was performed on formalin-fixed, paraffin-embedded sections using the Envision Plus detection system (Dako, Carpinteria, CA). Eleven carcinomas with *NUT* rearrangements (see Results) were stained for keratin antibodies AE1/AE3 (keratins 1 to 8, 10, 14 to 16, and 19; Dako), CAM 5.2 (keratins 8, 18, and 19; Becton Dickinson, San Jose, CA), PanK (keratins 5, 6, 8, 17, and 18; clone MNF116, Dako), CK7 (keratin 7, n = 7 patients tested; clone OV-TL12/30, Dako) or CK20 (keratin 20, n = 7 patients tested; clone Ks20.8, Dako), placental alkaline phosphatase (Dako), and CD34 (clone Qbend10; Immunotech, Marseille, France). A comparison group of 45 poorly differentiated malignant tumors lacking *NUT* rearrangements was stained

for CD34. This comparison group included poorly differentiated carcinoma (n = 14), poorly differentiated squamous cell carcinoma (n = 8), lymphoepithelioma (n = 5), poorly differentiated adenocarcinoma (n = 4), poorly differentiated neuroendocrine carcinoma (n = 3), embryonal cell carcinoma (n = 3), poorly differentiated germ cell tumor (n = 2), poorly differentiated mucoepidermoid carcinoma (n = 2), poorly differentiated malignant neoplasm, not otherwise specified (n = 2), and high-grade transitional-cell carcinoma (n = 2).

RESULTS

The FISH studies revealed *NUT* rearrangement in 11 patients. Seven of these were new unpublished cases from the group of 98 screened tumors (7.1%). Only one of the *NUT*-rearranged tumors from the screened group, a patient with the t(15;19), had karyotyping performed before selection for this study. The clinicopathologic characteristics of the 98 patients with tumors who were screened are listed in Table 1. Most patients were young (median age, 32.5 years) and often had poorly differentiated carcinomas (43%). Primary sites were entirely from the head, neck, or trunk, predominantly midline (73%), and were frequently within the respiratory tract (47%).

Clinicopathologic features of the 11 patients with tumors with *NUT* break points are listed in Table 2. All *NUT*-rearranged tumors were carcinomas, as defined by squamous differentiation or expression of epithelial keratins (Table 2). In contrast to the *NUT* wild-type (*NUT*wt) group of tumors (n = 91; median age, 33.4 years), *NUT*-rearranged tumors were diagnosed exclusively in adolescents and young adults (median age, 17.6 years). Notably, our FISH screening method revealed the first case of *BRD4-NUT*–rearranged carcinoma in a toddler (age, 3 years), who succumbed rapidly despite chemotherapy and radiation therapy, with adrenal and renal metastases from a bladder primary (patient 4). Eight of the NRCs also harbored the *BRD4* gene break point, which is consistent with *BRD4-NUT* fusion. Three patients harbored the *NUT* but not *BRD4* translocation break point, which identified, for the first time, the existence of variant rearrangements involving *NUT*.

Histologic features of NRCs (Fig 1A, 1B, and 1D) included varying degrees of squamous differentiation (n = 9, 82%) and absence of glandular differentiation (n = 11). All *BRD4-NUT* carcinomas were poorly differentiated, whereas the *NUT*-variant carcinomas had more pronounced squamous differentiation. All *NUT*-variant carcinomas had multifocal keratinization, and one (patient 9) was extensively keratinized (Fig 1B), warranting the diagnosis of well-differentiated squamous cell carcinoma. As a group, NRCs showed considerable morphologic overlap with typical *NUT*wt squamous cell carcinomas (Fig 1C).

All NRCs involved midline structures, including sinus/ orbit, nasopharynx, trachea, thymus, mediastinum, lung,

Table 1. Patient Characteristics

Characteristic	No. of Patients (N = 98)	%
Sex		
Male	53	53.6
Female	45	46.4
Age, years		
Median	32.5	
Range	0-76	
Diagnosis		
Poorly differentiated ca	28	28.6
Squamous cell ca*	19	19.4
Other†	15	15.3
Lymphoepithelioma	11	11.2
Germ cell tumor‡	8	8.2
Non–small–cell ca, NOS	5	5.1
Adenocarcinoma	5	5.1
Neuroendocrine ca	4	4.1
Thymic ca§	3	3.1
Primary site		
Lung	26	26.5
Lymph node	14	14.3
Nasopharynx/parapharynx	9	9.2
Other‖	7	7.1
Head and neck	5	5.1
Bronchus	5	5.1
Mediastinum	5	5.1
Testis	5	5.1
Sinonasal	5	5.1
Thymus	3	3.1
Parotid	3	3.1
Tongue	3	3.1
Bladder	3	3.1
Trachea	2	3.1
Thyroid	2	2.0
Orbit	1	1.0

Abbreviations: ca, carcinoma; NOS, not otherwise specified.

*Includes well differentiated (n = 1), moderately differentiated (n = 2), poorly differentiated (n = 15), and NOS (n = 1).

†Includes transitional-cell carcinoma (n = 2), acinic-cell carcinoma (n = 1), small-cell carcinoma (n = 1), papillary thyroid carcinoma (n = 2), olfactory neuroblastoma (n = 3), malignant pleural mesothelioma (n = 1), malignant neoplasm NOS (n = 3), mucoepidermoid carcinoma (n = 1), and spindle-cell sarcoma (n = 1).

‡Includes seminoma (n = 2) and embryonal carcinoma (n = 6).

§Type C thymoma (WHO classification).

‖Includes esophagus (n = 1), chest wall (n = 1), pleura (n = 1), breast (n = 1), abdomen (n = 2), and endometrium (n = 1).

and bladder. No sex predilection was seen. All patients (except patient 10, *NUT* variant) succumbed to disease despite aggressive treatment with multimodality intensive chemotherapies and/or radiation therapy. The average survival time for *NUT*-variant carcinomas was 96.3 weeks, almost four-fold greater than for *BRD4-NUT* carcinomas (28 weeks; $P < .0001$). All 11 NRCs developed hematogenous metastases, although lymphatic spread was seen only in *NUT*-variant carcinomas. Despite these apparent clinical differences, there was no difference in mitotic counts between the *BRD4-NUT* (10.5 mitoses/10 hpf) and *NUT*-variant (12.7 mitoses/10 hpf) carcinomas.

To evaluate the possibility that NRCs have distinctive phenotypic features, additional immunohistochemical studies were performed (Table 2). The results were notable for the frequent (seven of 11 tumors, 64%) expression of the stem-cell and vascular marker CD34 on NRCs. This was in contrast to the nonreactivity (n = 43) or, at most, focal weak reactivity (n = 2) of *NUT*wt neoplasms for CD34. In contrast, stains for placental alkaline phosphatase, a sensitive marker for germ cell tumors, were completely negative in 10 of 11 *NUT*-rearranged tumors, with the remaining tumor showing only weak focal staining. Most NRCs were reactive for CK7 (six of seven tumors). CK20 immunostaining was either negative (two of seven tumors) or showed only focal reactivity (five of seven tumors).

DISCUSSION

The midline carcinoma with *NUT* rearrangement is the only known carcinoma defined by its molecular signature. The consistency of the *NUT* break point is herein demonstrated in the largest series (11 patients) reported to date, underscoring the molecular genetic uniformity of these tumors.

An unexpected and important finding in this series of NRCs is the discovery of tumors with *NUT*-variant rearrangements, in which *NUT* is fused apparently with an oncogene other than *BRD4*. Like tumors with the *BRD4-NUT*, all cases with *NUT*-variant rearrangement(s) were carcinomas. However, our preliminary evidence suggests that *NUT*-variant carcinomas are histologically better differentiated; clinically, the pattern of tumor spread differed from *BRD4-NUT* carcinomas, and patients lived almost four-fold longer. The significance of these differences has yet to be determined in a larger series; nevertheless, there may be a critical prognostic difference between *BRD4-NUT* and *NUT*-variant tumors.

We hypothesize that, like t(15;19), the t(15;variant) results in formation of a fusion oncogene between *NUT* and an unknown partner gene. It is possible that the variant partner gene encodes a bromodomain protein similar to BRD4. The *BRD4-NUT* fusion oncogene contains a promoter that results in ubiquitous expression and two bromodomains of *BRD4* capable of binding chromatin constitutively.[10,11] We hypothesize that the *BRD4* promoter and bromodomains drive aberrant *NUT* expression and chromatin binding. The variant partner gene may also serve these functions and, thus, belong to a similar family as *BRD4*.

Our discovery of the *NUT*-variant subgroup establishes the *NUT* oncogene as the common denominator in midline carcinoma of young people, and we propose that such carcinomas (whether having *BRD4-NUT* or *NUT*-variant rearrangements) be designated as midline carcinoma with *NUT* rearrangement. The *NUT*-variant subgroup is also relevant in demonstrating that these carcinomas can arise

Table 2. Clinicopathologic Characteristics of Carcinomas With *NUT* Rearrangement

Patient	t(15)	t(19)	Age (years)	Sex	Primary Site	Diagnosis	Sq Diff*	Rx	Metastasis	Survival (weeks)	Keratins†	PLAP	CD34	Reference
1	Pos	Pos	22	F	Thymus	PD ca	+	CR	Bone, lung, pleural fluid	14	+‡	−	−	Kubonishi et al[1]
2	Pos	Pos	13	F	Epiglottis	Undiff ca	−	CR	Lymph node, skin	36	+	−	+	Vargas et al[5]§
3	Pos	Pos	12	F	Nasopharynx	PD sq cell ca	+	CR	Bone	13	+	−	+	Vargas et al,[5]‖ French et al[6]
4	Pos	Pos	15	M	Mediastinum	Thymic PD sq cell ca	++	C	Lung, bone, pleural fluid	24	+	−	−	Toretsky et al[7]
5	Pos	Pos	3	M	Bladder	PD sq cell ca	+	CR	Kidney, adrenal	34	+	−	+	
6	Pos	Pos	15	F	Orbit	PD sq cell ca	+	CR	NA	28	+	−	+	
7	Pos	Pos	26	M	Sinonasal	Undiff ca	−	CR	Bone	67	+	−	+	
8	Pos	Pos	35	F	Mediastinum	PD ca	+	R	Bone, soft tissue, pleura	8	+	−	+	
9	Pos	Neg	16	M	Lung	Sq cell ca	+++	CR	Bone, lymph node, skin	148	ND	−	−	
10	Pos	Neg	16	F	Trachea	PD ca	++	CR	Lymph node, bone	ALWD 100	+	+	+	
11	Pos	Neg	21	F	Nasopharynx	Nasopharyngeal ca	++	CR	Bone, lymph node, skin	41	+	−	−	

Abbreviations: Sq, squamous; Diff, differentiated; Rx, treatment; PLAP, placental alkaline phosphatase; Pos, positive; PD, poorly differentiated; ca, carcinoma; CR, chemotherapy and radiotherapy; Undiff, undifferentiated; C, chemotherapy; NA, not available; R, radiotherapy; Neg, negative; ND, not determined; ALWD, alive with disease.

*+, very focal squamous differentiation (determined by electron microscopy or presence of keratinization); ++, moderately focal keratinization; +++, extensive keratinization; −, no keratinization.

†Includes one or more of the following antibodies: AE1/AE3, Pan K, CAM 5.2.

‡+, reactivity to specified antibody; −, nonreactivity to specified antibody.

§Patient 1.

‖Patient 2.

without rearrangement of the chromosome 19p13.1 region containing *NOTCH3*. It has been hypothesized that *NOTCH3*, because of its proximity to the chromosome 19p13.1 break point, is the oncogene responsible for the t(15;19) carcinoma.[4] Because the 19p13.1 break point is lacking in variant carcinomas with *NUT* rearrangement, it is unlikely that *NOTCH3* plays a critical role in the t(15;19) carcinoma, where a *BRD4-NUT* fusion oncogene is expressed.

Although t(15;19) carcinomas were previously hypothesized to originate from respiratory tract or thymus, our finding of a bladder carcinoma with *BRD4-NUT* suggests that NRCs can arise from various midline epithelial surfaces. It is possible that NRCs arise from early epithelial progenitor-cell rests that are greatest in number during the first two to three decades of life. The CD34 reactivity might also be relevant, in that some epithelial cell precursors, including hair follicle and hepatobiliary stem-like cells, express CD34,[12,13,14] a marker previously associated with hematopoietic stem cells; vascular endothelium and soft tissue tumors (angiomyolipoma, gastrointestinal stromal tumors, and some neural and fibrous tumors) are also reactive for CD34. If *NUT* carcinomas indeed arise from early epithelial stem cells, they might contain a large percentage of self-renewing cells, accounting for their distinctly aggressive biology. However, this hypothesis will need to be evaluated formally because poorly differentiated tumors are known to express antigens uncharacteristic for their supposed lineage and which have no obvious relevance to their biology. Although the biologic significance of CD34 reactivity is unclear, our findings show that it is a specific marker for carcinomas harboring the *NUT* rearrangements and should be a helpful adjunct in identifying future cases for both clinical and investigative purposes.

The most consistent clinical findings described in previous reports and confirmed in this series of NRCs are the young ages of the patients and the aggressive, rapidly lethal nature of tumors with the *BRD4-NUT* fusion. Therefore, the *BRD4-NUT* fusion conveys important prognostic information with potential therapeutic relevance, underscoring the importance of assaying poorly differentiated squamous carcinomas in young people for the t(15;19).

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Authors' Disclosures of Potential Conflicts of Interest

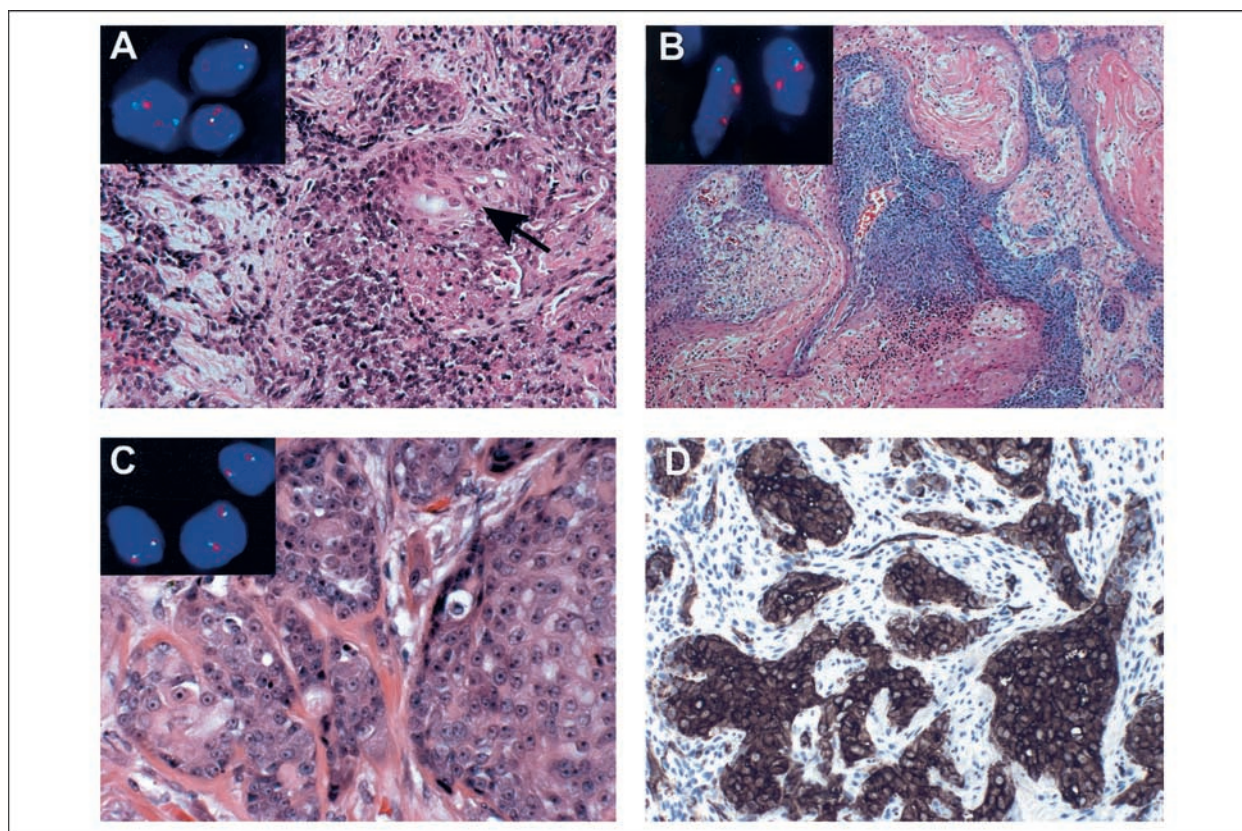The authors indicated no potential conflicts of interest.

Fig 1. Histology and genetic alterations. (A) Patient 5, with poorly differentiated *BRD4-NUT* bladder tumor, has focal keratinization (arrow) and *NUT* rearrangement by dual-color, split-apart fluorescence in situ hybridization (inset). (B) Patient 9, with *NUT*-variant lung primary tumor, has extensive keratinization and *NUT* rearrangement (inset). (C) *NUT* wild-type squamous cell carcinoma lacks *NUT* rearrangement (inset). (D) CD34 staining of NUT-variant tracheal tumor in patient 10.

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