**BRD4-NUT Fusion Oncogene: A Novel Mechanism in Aggressive Carcinoma**

Christopher A. French, Isao Miyoshi, Ichiro Kubonishi, Holcombe E. Grier, Antonio R. Perez-Atayde, and Jonathan A. Fletcher

**Abstract**

The poorly differentiated carcinoma with t(15;19)(q13, p13.1) is characterized by its highly aggressive, invariably lethal clinical course. The chromosome 19 translocation breakpoint targets the **BRD4** double bromodomain-containing gene, which functions in regulation of cell cycle progression. Herein we demonstrate that **BRD4** is fused with nearly the entire transcript of the novel 15q13 gene, **NUT** (nuclear protein in testis), forming a 6.4-kb fusion oncogene, **BRD4-NUT**. **NUT**, like **BRD4**, is predicted to encode a nuclear protein but, unlike the ubiquitous **BRD4** transcript, is expressed only in testis. These findings establish a model to elucidate the oncogenic consequences of unscheduled **NUT** expression and altered **BRD4** function. Very few fusion oncogenes have been identified in epithelial tumors, and **BRD4-NUT** is the first fusion oncogene mechanism identified in a highly lethal form of carcinoma.

**Introduction**

Translocation t(15;19)(q13, p13.1) characterizes a rare, aggressive, and lethal carcinoma arising in midline organs of young people. The translocation results in a heretofore uncharacterized fusion oncogene. As described previously (1), we have determined that the chromosome 19 translocation breakpoint targets the **BRD4** bromodomain gene, whereas the chromosome 15 breakpoint involves a 9-kb region on chromosome band 15q13. **BRD4** is expressed normally as two alternative transcripts with identical 5' ends; but the coding sequence of the longer **BRD4** transcript is approximately twice the length of the shorter transcript. Both **BRD4** transcripts encode the NH2-terminal bromodomains, whereas the longer **BRD4** transcript encodes COOH-terminal lysine-rich regions that are not encoded by the shorter **BRD4** transcript. Notably, the t(15;19) translocation breakpoint transsects the coding sequence of the longer **BRD4** gene, whereas the shorter **BRD4** transcript is unperturbed.

**In vitro** (2, 3) and **in vivo** (4) studies of the murine **BRD4** have revealed a critical role in the regulation of cell cycle progression and cellular proliferation. **BRD4** associates with chromatin (3) and binds replication factor C (RFC; Ref. 2). Notably, whereas **BRD4** expression regulates G2-M transition, **BRD4** overexpression inhibits G1-S phase transition. In addition, **in vivo** studies (4) suggest a pivotal role for **BRD4** in cellular proliferation during embryogenesis.

To fully characterize the molecular mechanism of oncogenesis in t(15;19)-associated carcinomas, we undertook mapping and cloning of the chromosome 15 translocation target. Herein, we demonstrate that the chromosome 15 translocation rearranges the novel gene, **NUT**, resulting in a **BRD4-NUT** fusion oncogene. We also report expression profiles for **NUT** and **BRD4** in normal tissues. These studies reveal the first known fusion oncogene in a highly malignant form of epithelial neoplasia.

**Materials and Methods**

**Electronic Sequence Analysis.** Human ESTs in the 15q13 translocation breakpoint (Nop10p region) were identified using the Human Genome Browser (UCSC). Sequence analyses were performed using BLAST [National Center for Biotechnology Information (NCBI)].

**Cell Lines.** We established two rapidly growing, immortal cell lines from t(15;19) carcinomas, both of which have been reported previously (5, 1). The chromosome bands have been cytogenetically stable, with persistence of the t(15;19) translocation in all cells after more than 20 passages.

**RNA Isolation and RT-PCR.** Polyadenylate-enriched RNA was isolated using Micro FastTrack (Invitrogen Corporation, Carlsbad, CA). RT-PCR was performed using the SMART RACE Kit (Clontech Laboratories, Inc., Palo Alto, CA) according to the manufacturer’s instructions, with first-strand synthesis using SMART adapter primers. **BRD4-NUT** fusion cDNA was then amplified by nested PCR using ExTaQ (Takara Bio Inc., Otsu, Shiga, Japan). Primers were chosen using the Whitehead Genome Center Primer3 software, and all of the primers were numbered according to **BRD4** and **NUT** cDNA sequences (as per GenBank accession nos. AF368649 and AF482429, respectively). First-round primers were BR2276F (AAGTGATGTTAGTTGCCC-GCTCCTC) and NUT1194R (GAGGTCTCTGGCTTTACGCTGACG), and second-round primers were BR2334F (GAGCCCTAGTGCAGTCGCTCTTG) and NUT1132R (GGAATGTACACTTGCGTGGCAGCA). Gel-purified nested PCR products were cycle sequenced by incorporation of ABI PRISM Big Dye Terminators (Perkin-Elmer, Inc., Wellesley, MA) and analyzed on an ABI 310 sequencer.

**Northern Blot Analysis.** Polyadenylate-enriched RNA was isolated from cultured cells using Micro FastTrack kit (Invitrogen), separated by electrophoresis through a formaldehyde-containing gel, and then transferred to a Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). The blot was hybridized with a 400-bp NUT cDNA probe. Details of the oligonucleotide primers are available by request. **Northern Blot Analysis.** Polyadenylate-enriched RNA was isolated from cultured cells using Micro FastTrack kit (Invitrogen), separated by electrophoresis through a formaldehyde-containing gel, and then transferred to a Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). The blot was hybridized with a 400-bp NUT cDNA probe. Details of the oligonucleotide primers are available by request. **Northern Blot Analysis.** Polyadenylate-enriched RNA was isolated from cultured cells using Micro FastTrack kit (Invitrogen), separated by electrophoresis through a formaldehyde-containing gel, and then transferred to a Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). The blot was hybridized with a 400-bp NUT cDNA probe. Details of the oligonucleotide primers are available by request. 

**Northern Blot Analysis.** Polyadenylate-enriched RNA was isolated from cultured cells using Micro FastTrack kit (Invitrogen), separated by electrophoresis through a formaldehyde-containing gel, and then transferred to a Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). The blot was hybridized with a 400-bp NUT cDNA probe. Details of the oligonucleotide primers are available by request. 

**Northern Blot Analysis.** Polyadenylate-enriched RNA was isolated from cultured cells using Micro FastTrack kit (Invitrogen), separated by electrophoresis through a formaldehyde-containing gel, and then transferred to a Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). The blot was hybridized with a 400-bp NUT cDNA probe. Details of the oligonucleotide primers are available by request. 

**Northern Blot Analysis.** Polyadenylate-enriched RNA was isolated from cultured cells using Micro FastTrack kit (Invitrogen), separated by electrophoresis through a formaldehyde-containing gel, and then transferred to a Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). The blot was hybridized with a 400-bp NUT cDNA probe. Details of the oligonucleotide primers are available by request. 

**Northern Blot Analysis.** Polyadenylate-enriched RNA was isolated from cultured cells using Micro FastTrack kit (Invitrogen), separated by electrophoresis through a formaldehyde-containing gel, and then transferred to a Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). The blot was hybridized with a 400-bp NUT cDNA probe. Details of the oligonucleotide primers are available by request.

**Acknowledgments.** This work was supported by NIH Institutional and National Research Service Award Grant T32-HL07627 and NIH National Cancer Institute Mentored Clinical Scientist Award K08 CA02158-01 (to C.A.F.).

**References.** 1. Supported by NIH Institutional National Research Service Award Grant T32-HL07627 and NIH National Cancer Institute Mentored Clinical Scientist Award K08 CA02158-01 (to C.A.F.). 2. To whom requests for reprints should be addressed, at Department of Pathology, Brigham and Women’s Hospital, 75 Francis Street, Boston, MA 02115. Phone: (617) 732-6490; Fax: (617) 264-6301; E-mail: cfrrench@partners.org.

**Received 11/7/02; accepted 12/2/02.**

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1. The abbreviations used are: EST, expressed sequence tag; MTN, multiple tissue Northern (blot); UCSC, University of California at Santa Cruz; BLAST, basic local alignment search tool; RT-PCR, reverse transcription-PCR; ET, extraterminal; CBP, Creb binding protein.

2. Internet address for detail: cfrrench@partners.org.
Results

Identification of an EST Flanking the Chromosome 15q13 Breakpoint. Our previously reported Southern blotting analyses (1) narrowed the chromosome band 15q13 breakpoint to a <9-kb region. We searched for genes in this region using the Human Genome Browser (Human Genome Project Working Draft, UCSC). Thereby, we identified a spliced EST (GenBank accession no. AL040312) whose sequence appeared to span the <9-kb translocation breakpoint region. BLAST analysis revealed that this EST overlapped a 2134-bp cDNA clone (GenBank accession No. AL133071) that, as ascertained by 6-frame translation Baylor College of Medicine (BCM) Search Launcher), comprised the 3' coding sequence of a novel gene. Because the eight known ESTs for this gene (GenBank) were from testis cDNA libraries and were also based on Northern blot evidence for testis-restricted expression (see below) and for the presence of typical nuclear localization signals (see below), we have preliminarily named this gene NUT (Nuclear Protein in Testis).

Identification of the BRD4-NUT Fusion Transcript. RT-PCR was performed empirically using NUT reverse primers and BRD4 forward primers (exon 10) predicted, based on our genomic localizations, to be 5' to the BRD4 translocation breakpoint. A 1-kb BRD4-NUT fusion product was amplified readily and reproducibly from each of two t(15;19)-positive cancers, whereas BRD4-NUT RT-PCR products were not obtained from t(15;19)-negative control cDNAs (Fig. 1A). Sequencing revealed that the BRD4-NUT RT-PCR products in both of the t(15;19)-positive tumors were identical, containing in-frame fusion transcripts in which BRD4 exon 10 is fused to NUT exon 2.

RT-PCR using numerous nested forward and reverse primer pairs failed to amplify a NUT-BRD4 product from either of the t(15;19)-positive carcinoma cell lines.

BRD4-NUT, BRD4, and NUT Gene Structures. The t(15;19) translocation breakpoint bisects the BRD4 longer transcript into components encoding amino acids 1–720 and 721–1372. The NH2-terminal component (Fig. 1B) contains the BRD4 bromodomains and, therefore, might contribute chromatin-binding and potential coactivation functions (6) to the BRD4-NUT fusion oncoprotein. Other BRD4 domains in BRD4-NUT are less well characterized. These include a potential kinase domain (3), an ET protein-protein interaction domain (7), and a serine-rich potential transactivation or corepressor domain (Ref. 8; Fig. 1B).

The chromosome 15 translocation breakpoint separates NUT exon 1 (potentially encoding amino acids 1–5) from exons 2–7 (encoding amino acids 6–1127). Hence, almost the entire NUT coding sequence is contained in the BRD4-NUT fusion transcript. NUT (GenBank accession no. AF482429) is predicted to encode a Mr 120,000 nuclear protein with 64% homology to a novel Mr 66,800 protein encoded by a locus on chromosome 10.

Expression of BRD4-NUT. NUT expression was evaluated by Northern blot analysis in a t(15;19)-positive carcinoma cell line, normal human testis, and in cell lines established from other malignant tumors (Fig. 2A). A 6.4-kb putative BRD4-NUT transcript was expressed in the t(15;19)-positive carcinoma cell line, and a 3.6-kb wild-type NUT transcript was expressed in normal testis. NUT ex-

---

**Fig. 1. A**, RT-PCR demonstrates identical BRD4-NUT fusion transcripts in t(15;19)-positive carcinoma cell lines, TY82 and UC143. Control reactions were performed using cDNAs from NHL548 (non-Hodgkin’s lymphoma), LC448 (lung carcinoma), total placenta, and no template. B, predicted BRD4-NUT protein sequence includes the two bromodomains, ET domain, and serine-rich potential transactivation or corepressor domain (Ref. 8; Fig. 1B).

---

**Fig. 2. A**, BRD4-NUT expression was evaluated by Northern blot analysis in a t(15;19)-positive carcinoma cell line, normal human testis, and in cell lines established from other malignant tumors (Fig. 2A). A 6.4-kb putative BRD4-NUT transcript was expressed in the t(15;19)-positive carcinoma cell line, and a 3.6-kb wild-type NUT transcript was expressed in normal testis. NUT ex-

---

**Fig. 3.** A, RT-PCR demonstrates identical BRD4-NUT fusion transcripts in t(15;19)-positive carcinoma cell lines, TY82 and UC143. Control reactions were performed using cDNAs from NHL548 (non-Hodgkin’s lymphoma), LC448 (lung carcinoma), total placenta, and no template. B, predicted BRD4-NUT protein sequence includes the two bromodomains, ET domain, and serine-rich domain from BRD4, and almost the entire NUT sequence. The bipartite nuclear localization sequences (NLS) are preserved in the BRD4 component, and an additional putative NLS is contributed by NUT.
BRD4 transcripts are expressed variably in all of the samples. A 6.4-kb aberrant NUT transcript is seen in TY82, and a normal 3.6-kb NUT transcript is demonstrated only in testis. Therefore, we conclude that BRD4-NUT is the functional fusion oncogene associated with the t(15;19) translocation. Although the oncogenic mechanisms of BRD4-NUT have not yet been determined, the t(15;19) is highly characteristic of a clinically distinctive cancer, and it is likely that BRD4-NUT plays a pivotal pathogenetic role in this extremely lethal form of carcinoma.

Several aspects of these findings are unique and intriguing. The t(15;19)-positive poorly differentiated carcinoma is one of very few epithelial neoplasms that harbor defining translocations (9). Among this limited group, the t(15;19)-positive carcinoma is by far the most clinically aggressive. Most patients die within 3 months (1, 10). Therefore, this cancer provides one of the few models with which to study (a) the role of fusion oncogene mechanisms in epithelial neoplasia, and (b) oncogenic mechanisms of clinically aggressive neoplasia. In particular, the transforming mechanisms of bromodomain oncoproteins, although studied recently in hematological neoplasia (11), have not yet been evaluated in solid tumors.

Although the clinical ramifications of BRD4-NUT have not been determined in systematic studies, all of the known t(15;19)-positive carcinomas were rapidly metastasizing and extremely lethal. Therefore, we expect that diagnostic recognition of BRD4-NUT will be useful in identifying this clinically aggressive subset of carcinomas. Presumably, individuals with t(15;19)-positive carcinoma require immediate treatment with intensive systemic therapies to attempt ablation of their rapidly progressive disease. Such therapeutic efforts will be enabled by prompt molecular diagnosis of the BRD4-NUT mechanism. The clinical diagnosis of BRD4-NUT oncogenes can be accomplished by several methods. The translocation t(15;19) can be demonstrated in fresh clinical specimens by conventional karyotyping, as has been reported by several groups (10, 12–15). BRD4-NUT genomic rearrangement can...
also be demonstrated, including in frozen or formalin-fixed spec-
imens, by fluorescence in situ hybridization (1). Although not yet
evaluated in clinical specimens, we have shown herein that the
BRD4-NUT genomic rearrangement can be determined at the tran-
script level by RT-PCR. Furthermore, it is conceivable that NUT
immunohistochemistry might provide a simple alternative to mo-
lecular detection, given that NUT expression has been identified
thus far only in normal testis and t(15;19)-positive carcinoma.

The COOH-terminal end of BRD4-NUT incorporates almost the
entire NUT sequence. Given that NUT expression is restricted to
the testis, it is likely that unscheduled NUT expression in the t(15;19)-
positive carcinomas results from oncogenic juxtaposition to BRD4,
with expression then being regulated by the BRD4 promoter elements.
NUT oncogenic function likely results both from ectopic expression
in an epithelial cell lineage and from the structural consequences
of fusion to BRD4. Expression of NUT protein, therefore, may be a
specific marker of the t(15;19) carcinoma, as well as a potential
therapeutic target.

BRD4 represents the first known oncogene from the BET family of
bromodomain genes, which are defined by the presence of NH2-
terminal bromodomain(s) and an ET domain (7). The intact bromo-
domain regions and the ET domain are contained in the BRD4
component of the BRD4-NUT fusion oncoprotein. The second bro-
domain of BRD4 has been shown to directly bind replication factor
C, a multi-subunit complex essential for DNA replication, and
through this interaction, inhibits G1-S phase transition when overex-
pressed (2).

Given the known functions of the longer BRD4 isoform, and the
ubiquitous presence of the biologically uncharacterized short isoform,
the oncogenic BRD4 mechanisms are likely to be complex in the
t(15;19) carcinoma. One scenario that warrants evaluation is that the
BRD4 short and long isoforms might have qualitatively different, and
even opposing, functions in cell cycle regulation. Such a mechanism
could be relevant in the t(15;19) carcinomas, in which the transloca-
tion transacts the coding sequence of the BRD4 long isoform, without
affecting that of the short isoform. These considerations suggest that
the t(15;19) translocation breakpoint might abrogate BRD4 long iso-
form function, and it is significant that the BRD4 component in the
BRD4-NUT fusion protein comprises virtually all of the predicted
BRD4 short isoform. Therefore, the BRD4-NUT oncoprotein could
function as a dominant negative in relationship to the BRD4 long
isoform, and might also contribute a gain-of-function equivalent of the
BRD4 short isoform. Notably, the BRD4 short and long isoforms are
expected to compete for binding to chromatin, given that their bro-
modomain-containing NH2-terminal regions are identical. Conse-
quences of BRD4-NUT fusion that might contribute to transforming
function could, therefore, include: (a) reduced expression of BRD4
long isoform; (b) increased avidity of BRD4-NUT in chromatin
binding, resulting in functional inhibition of the non-fusion BRD4
isoforms; and (c) other altered BRD4 functions, e.g., perturbed inter-
actions with corepressors/coactivators, possibly resulting from the
NUT component of the fusion protein.

Although BRD4-NUT is the first example of a bromodomain-
containing oncogene in a solid tumor, bromodomain-containing
fusion oncoproteins have been characterized in several types of
leukemia. For example, the CBF bromodomain-containing protein
is rearranged in acute myelogenous leukemia with t(8;16) translo-
cation (11), resulting in a fusion oncogene. MOZ is a transactivat-
ing component of the MOZ-CBP complex (16), which is thought to
regulate transcription of genes that influence myeloid differentia-
tion programs. In contrast, the MOZ-CBP fusion oncoprotein in-
hibits AML1-mediated transcription and thereby creates a leuko-
emic differentiation block through dominant-negative effects on AML1 function (16). By analogy, the BRD4-NUT fusion oncoprotein might function in part via perturbed interac-
tions between NUT and NUT-binding proteins. This possibility
seems particularly likely in that nearly the entire NUT sequence is
included in BRD4-NUT. However, the model of BRD4-NUT in
the t(15;19) carcinoma differs from that of MOZ-CBP in leukemia,
because MOZ-CBP fusion alters the function of proteins that are
normally expressed in the transformed cell lineage. In the case of
BRD4-NUT, there are presently no clues (other than nuclear loca-
localization) as to the normal function of NUT, and there is no
evidence that non-oncogenic NUT plays important functional
roles, or is even expressed, in epithelial cell lineages.

References
1. French, C. A., Miyoshi, I., Astor, J. C., Kubonishi, I., Kroll, T. G., Dal Cin, P.,
Vargas, S. O., Perez-Atayde, A. R., and Fletcher, J. A. BRD4 bromodomain gene
rearrangement in aggressive carcinoma with translocation t(15;19). Am. J. Pathol.,
2. Maruyama, T., Farina, A., Dey, A., Cheong, J., Bermudez, V. P., Tamura, T.,
Sciortino, S., Shuman, J., Hurwitz, J., and Ozato, K. A Mammalian bromodomain
protein, brd4, interacts with replication factor C and inhibits progression to S phase.
3. Dey, A., Ellenberg, J., Farina, A., Coleman, A. E., Maruyama, T., Sciortino, S.,
Lippincott-Schwartz, J., and Ozato, K. A bromodomain protein. MCAP, associates
and Bedddington, R. S. Growth and early postimplantation defects in mice defi-
3802, 2002.
5. Kuzumae, T., Kubonishi, I., Takeuchi, S., Takeuchi, T., Iwata, J., Sonobe, H., Ohtsuki,
Y., and Miyoshi I. Establishment and characterization of a thymic carcinoma cell line
(Ty-82) carrying t(15;19)(q15;p13) chromosome abnormality. Int. J. Cancer, 50:
7. Florence, B., and Fuller, D. V. You betcha: a novel family of transcriptional
the transcriptional, antipapotic, and transforming activities of the v-Rel oncoprotein.
Bioscience, 2002.
10. Vargas, S. O., French, C. A., Faul, P. N., Fletcher, J. A., Davis, I. J., Dal Cin, P., and
Perez-Atayde, A. R. Upper respiratory tract carcinoma with chromosomal transloca-
tion 15;19. Evidence for a distinct disease entity of young patients with a rapidly fatal
G. C., Dobner, H., Jotterand-Bellomo, M., Falkenburg, J. H., Slater, R. M., van
of CBP rearrangements in acute myelogenous leukemia with t(8;16). Leukemia
I. Novel t(15;19)(q15;p13) chromosome abnormality in a thymic carcinoma. Cancer
13. Lee, A. C., Kwong, Y. I., Fu, K. H., Chan, G. C., Ma, L., and Lau, Y. L. Disseminated
Roberts, J. R., and Carbone, D. P. Chromosome 19 translocation, over-expression of
of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion
**BRD4-NUT** Fusion Oncogene: A Novel Mechanism in Aggressive Carcinoma

Christopher A. French, Isao Miyoshi, Ichiro Kubonishi, et al.