Truncated DNMT3B Isoform DNMT3B7 Suppresses Growth, Induces Differentiation, and Alters DNA Methylation in Human Neuroblastoma

Kelly R. Ostler1, Qiwei Yang2,5, Timothy J. Looney3, Li Zhang3, Aparna Vasanthakumar1, Yufeng Tian2, Masha Kocherginsky4, Stacey L. Raimondi1,6, Jessica G. DeMaio1,6, Helen R. Salwen2, Song Gu2,7, Alexandre Chlenski2, Arlene Naranjo4, Amy Gill2, Radhika Peddinti6, Bruce T. Lahn5, Susan L. Cohn2, and Lucy A. Godley1

Abstract

Epigenetic changes in pediatric neuroblastoma may contribute to the aggressive pathophysiology of this disease, but little is known about the basis for such changes. In this study, we examined a role for the DNA methyltransferase DNMT3B, in particular, the truncated isoform DNMT3B7, which is generated frequently in cancer. To investigate if aberrant DNMT3B transcripts alter DNA methylation, gene expression, and phenotypic character in neuroblastoma, we measured DNMT3B expression in primary tumors. Higher levels of DNMT3B7 were detected in differentiated ganglioneuroblastomas compared to undifferentiated neuroblastomas, suggesting that expression of DNMT3B7 may induce a less aggressive clinical phenotype. To test this hypothesis, we investigated the effects of enforced DNMT3B7 expression in neuroblastoma cells, finding a significant inhibition of cell proliferation in vitro and angiogenesis and tumor growth in vivo. DNMT3B7-positive cells had higher levels of total genomic methylation and a dramatic decrease in expression of the FOS and JUN family members that comprise API transcription factors. Consistent with an established antagonistic relationship between API expression and retinoic acid receptor activity, increased differentiation was seen in the DNMT3B7-expressing neuroblastoma cells following treatment with all-trans retinoic acid (ATRA) compared to controls. Our results indicate that DNMT3B7 modifies the epigenome in neuroblastoma cells to induce changes in gene expression, inhibit tumor growth, and increase sensitivity to ATRA. Cancer Res; 72(18); 1–10. © 2012 AACR

Introduction

In adult cancers, epigenetic changes and aberrant splicing of DNMT3B are observed frequently (1, 2). Pediatric neuroblastoma is characterized by a range of clinical behaviors, and genetic and epigenetic aberrations contribute to pathogenesis (3–5). The Cohn laboratory and others have shown that hypermethylation and silencing of genes involved in the regulation of tumor growth, cell cycle, apoptosis, and DNA repair are associated with aggressive growth and poor outcome in patients (6–8). Further, poor prognosis can be predicted by a CpG island methylator phenotype (9). Preclinical studies have demonstrated that neuroblastoma growth can be inhibited by drugs that disrupt DNA methylation (10, 11). Retinoic acid, a differentiation-inducing agent that has been shown to improve clinical outcome (12), can reverse the methylation status of hundreds of gene promoters (13), possibly due to decreased levels of the DNA methyltransferase (DNMT) enzymes (14).

Three DNMT enzymes regulate DNA methylation in eukaryotic cells (15–17), with widespread aberrant DNMT3B transcription in cancer cells, some encoding truncated proteins lacking the catalytic domain (1, 2, 18–20). Forced expression of DNMT3B7 within 293 cells led to altered DNA methylation and gene expression changes, indicating that DNMT3B7 expression could alter DNA methylation levels and gene expression (18). DNMT3B7 transgenic mice exhibit altered embryonic development (21).

Despite advances in genome-wide profiling that demonstrate strong correlations between genetic aberrations and clinical phenotype, we know much less about the clinical significance of epigenetic changes in neuroblastoma. Given that aberrant DNMT3B isoforms are common in cancer cells,
we hypothesized that aberrant *DNMT3B* transcripts found in neuroblastoma tumors could alter DNA methylation, gene expression, and tumor phenotype.

Materials and Methods

**Patients and tumor specimens**

Two ganglioneuroblastoma, three ganglioneuroma, and fourteen primary neuroblastoma tumors were obtained from Children’s Memorial Hospital in Chicago under an IRB approved protocol. Twelve RNA samples derived from one ganglioneuroblastoma and eleven neuroblastomas were obtained from the Children’s Oncology Group (COG) Neuroblastoma Tumor Bank. The laboratory studies were approved by The University of Chicago Institutional Review Board.

**Cell culture, cell identification, and nucleic acid isolation**

Cell line identities were verified by short tandem repeat profiling using the AmpF/STR Identifier PCR Amplification Kit (Applied Biosystems). RNA and DNA isolation was performed using TRIzol (Invitrogen) and Puregene Core Kit (Qiagen), respectively.

The MSCV-I-GFP plasmid backbone was engineered to confer constitutive *DNMT3B7* expression using a 5′ EcoRI site, C-terminal His-tag epitope, and 3′ *XhoI* site using primers listed in Supplementary Table S1 to ligate packaging plasmid psi-Eco were used to produce retroviral supernatants by 293T cell cotransfection using Fugene6 Transfection Reagent (Roche).

The inductive *DNMT3B7* construct was generated using oligonucleotides listed in Supplementary Table S1 to ligate into the pRetroX-Tight-Pur response vector (pRXTPL, Clontech Laboratories). The Tet-Off System (Clontech) was used to produce inducible LA1-55n cell lines. In the presence of doxycycline, there is no induction of gene expression in either the control or *DNMT3B7*-containing cell line. In the absence of doxycycline, the *DNMT3B7*-containing cells express both GFP and *DNMT3B7*, and the vector control cells express only GFP. We have compared the vector cells expressing GFP to the experimental cells expressing GFP and *DNMT3B7* to control for any effects of GFP expression. All plasmid inserts were sequenced to ensure proper correct sequence.

**Neuroblastoma xenograft studies**

Four- to six-week-old female homozygous athymic nude mice (Harlan) were inoculated subcutaneously into the right flank with *DNMT3B7*-expressing cells (or control) and measured twice weekly (see Supplementary Materials and Methods for details). Tumor volumes were calculated using the following formula: tumor volume = (length × width²)/2. Mice bearing SMS-KCNR (constitutive expression) xenografts were sacrificed after 28 days. Mice bearing LA1-55n (inducible expression) xenografts were divided randomly into two groups when palpable tumors developed: control mice continued receiving doxycycline-containing water to block *DNMT3B7* expression, and animals in the experimental group were given normal drinking water to induce *DNMT3B7* expression. Mice in both groups were sacrificed after 35 days.

**Immunohistochemical analysis**

Immunohistochemistry was performed for CD-31 (1:100, M-20, Santa Cruz Biotechnology) to determine the mean vascular density, and Ki-67 (1:200, MIB-1, DakoCytomation) to assess proliferation rate (10).

**Quantification of apoptosis**

*In situ* detection of apoptosis was performed using the *In Situ* Cell Death Detection Kit (Roche Diagnostics Corp.; ref. 22).

**Liquid chromatography/mass spectrometry**

Total cytosine methylation was performed by liquid chromatography/mass spectrometry (LC/MS), as described previously (21).

**Sodium bisulfite treatment, PCR amplification, and protein expression analysis**

Genomic DNA was treated with sodium bisulfite (23), and PCR amplifications were performed using the primers listed in Supplementary Table S1 using ZymoTaq (ZymoResearch) at the indicated temperatures. Reverse transcription was performed using Superscript III (Invitrogen), and PCR amplifications were performed using Platinum Taq (Invitrogen) using primers listed in Supplementary Table S1. Whole cell extracts were made by lysing cells in 75 mmol/L NaCl, 25 mmol/L Tris-CI, and 1:100 protease inhibitors (CalBiochem). *DNMT3B* was detected with T-16 antibody (1:500, sc-10236, Santa Cruz Biotechnology), and TOP1 was used as a loading control (1:400, ab3825, AbCam).

**RNA-Sequencing**

Total RNAs were isolated using Trizol Reagent (Invitrogen). RNA integrity was validated using the Agilent BioAnalyzer, and all samples had RNA integrity number >9. Libraries were generated following the Illumina protocol for Preparing Samples for Sequencing of mRNA. PCR amplified cDNA libraries were quantified on an Agilent 2100 Bioanalyzer. Single-end sequencing was performed for 36 cycles using Single Read Cluster Generation Kit V4 (Cat# GD-103-4001) and Sequencing Kits (Cat# FC-104-4002). Sequence reads from the RNA-sequencing (RNA-Seq) were aligned to genomic sequence (Human Feb. 2009 assembly, GRCh37/hg19). RNA-Seq data have been submitted to the GEO database, record number GSE36350.

**Statistical analyses**

Statistical analysis was performed using a 2-tailed Student’s *t* test or Fisher’ Exact Test. A *P* value ≤ 0.05 was considered statistically significant. For the RNA-Seq data, each *DNMT3B7*-expressing cell line was compared to the vector control and Bonferroni correction was used to correct for multiple testing using *n* = 18,674, the total number of genes that had at least one read in all 3-cell lines. Analysis of ATRA treatment of *DNMT3B7*-expressing or control cells was done using the mixed effect restricted maximum likelihood analysis model.
Results

Aberrant DNMT3B transcripts are expressed in primary neuroblastoma tumors

To investigate significance of aberrant DNMT3B transcripts in neuroblastoma, reverse transcriptase-PCR (RT-PCR) analysis was performed on a hypothesis-generating cohort of three ganglioneuroblastoma, three ganglioneuromas, and 25 primary neuroblastoma samples using primers that amplified all but 2 of the known alternative and aberrant DNMT3B isoforms (Fig. 1A, Supplementary Fig. S1A, Table 1). Additional DNMT3B transcripts were detected by Southern blot using a DNMT3B probe that hybridizes to all of the known aberrant isoforms amplified in the RT-PCR (Supplementary Fig. S1B and C). We stratified the patients into two risk groups: high risk and nonhigh risk. Nonhigh-risk patients included those with stages 1 and 2 disease, infants with stages 4 and 4S tumors, and patients with stage 3 tumors that lacked MYCN amplification. Similar to the criteria used by the COG, patients with stage 3 MYCN-amplified tumors and children older than 1 year of age with stage 4 disease were considered high risk (4). We found the expression of four or more aberrant DNMT3B isoforms correlated with higher risk groups \((P = 0.03)\). The identity of DNMT3B7 was confirmed by sequencing in four primary tumors (data not shown), and expression of DNMT3B7 was quantified in the primary tumors by real-time RT-PCR (Supplementary Fig. S2A). We obtained ten more ganglioneuroblastoma RNAs from the COG. However, the integrity of the RNA was too low to determine the levels of DNMT3B isoforms (data not shown). We therefore hypothesized that high levels of DNMT3B7 may contribute to the more benign clinical behavior of the most differentiated tumors.

Neuroblastoma cell lines showed expression patterns of DNMT3B transcripts similar to that of the primary tumors by RT-PCR (Fig. 1B). Tumorigenic N-type cells (24) had an increased number of DNMT3B transcripts and lacked DNMT3B7. By Western blot, we found the N-type cells have only full-length DNMT3B, whereas nontumorigenic S-type cells had both full-length DNMT3B and the truncated DNMT3B7 (Fig. 1C).

 Forced expression of DNMT3B7 in neuroblastoma cells inhibits growth

Because we detected high levels of DNMT3B7 in differentiated tumors associated with more favorable outcomes, we hypothesized that DNMT3B7 could modify neuroblastoma phenotype. We introduced DNMT3B7 into an N-type neuroblastoma cell line (LA1-55n) using a Tet-off inducible system. Repression of cell growth in the DNMT3B7-expressing cell lines was seen after 10 days of induction \((P < 0.001;\text{ Fig. 2A).}\) The expression of DNMT3B7 was evaluated by Western blot analysis at each time point (Fig. 2B). DNMT3B expression was low during the first week and increased as the growth of the cells decreased. The level of DNMT3B7-expression in the induced cells was two-fold higher than what was found in ganglioneuroblastoma tumors (Supplementary Fig. S2B). Similar results were obtained using constitutive expression of DNMT3B7 in another N-type neuroblastoma cell line, SMS-KCNR (Supplementary Fig. S3).

Both the inducible DNMT3B7-LA1-55n cells and constitutively-expressing DNMT3B7-SMS-KCNR cells were used to test the effects of DNMT3B7 expression on tumor growth in murine xenograft models using LA1-55n cells (Fig. 3A) and SMS-KCNR...
Table 1. DNMT3B transcripts expressed in primary ganglioneuroma, ganglioneuroblastoma, and neuroblastoma tumors

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>GNB</th>
<th>GN</th>
<th>Low risk</th>
<th>Intermediate risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3B2/3</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DNMT3B7</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>ΔDNMT3B7</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>ΔDNMT3B6</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Δ3B4/3B19</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Other</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Cells with DNMT3B7 expression have higher levels of global DNA methylation

We have shown previously that DNMT3B7 expression in an Eμ-Myc transgenic mouse model resulted in an increase in global DNA methylation (21). To determine if the expression of DNMT3B7 in the LA1-55n cells also increases DNA methylation, we measured global DNA methylation levels. As shown in Fig. 4A, significantly higher levels of DNA methylation were detected in the LA1-55n cells expressing DNMT3B7 as compared to vector control cells. We found the Satellite 2 repetitive elements had more DNA methylation following DNMT3B7-expression in the LA1-55n cells (Supplementary Fig S5). The increase in DNA methylation was also seen in both DNMT3B7-expressing xenografts. A statistically significant increase in total 5-methylcytosine levels was seen in the DNMT3B7-expressing xenografts compared to controls (P < 0.01, Fig. 4B), indicating that the pattern of DNA methylation is modified in the presence of the truncated DNMT3B7. Because we identified high levels of DNMT3B7 in ganglioneuroblastomas, we hypothesized that this would correlate with global DNA methylation levels. We found no difference between low, intermediate, and high-risk groups. However, there was a statistically significant increase in...
DNMT3B7 suppresses neuroblastoma growth

DNMT3B7 expression induces changes in gene expression and DNA methylation

To investigate the effects of DNMT3B7 on gene expression, we used RNA-Seq to compare the gene expression profiles of two independent DNMT3B7-expressing LA1-55n cells to vector control cells after 21 days of induction. After eliminating the genes with fewer than 150 reads per cell and those that had less than a three-fold change in either of the two DNMT3B7-expressing cell lines compared to the vector control, there were 144 genes that had at least an average five-fold change in the two DNMT3B7-expressing cell lines compared to the vector control cells (Supplementary Table S2). RNA-Seq confirmed the expression of DNMT3B7 only in the transduced cells (Supplementary Fig. S6). Similar to the expression of DNMT3B7 in Eμ-Myc transgenic mice (21), we found the expression of DNMT1, DNMT3A and endogenous DNMT3B was unchanged in the induced cells according to RNA-Seq, and real-time RT-PCR identified subtle increases in DNMT3A and DNMT3B (Supplementary Fig S7A to C). Among 117 genes with increased expression in the two DNMT3B7-expressing lines, 86 (73.3%) genes contained a CpG island, and 22 of the 27 (81.5%) of the genes with decreased expression contained a CpG island. Interestingly, eight of the 27 (29.6%) genes with decreased expression are found on chromosome 19 (P = 0.0068), and seven (25.9%) are on chromosome 19p (P < 0.0001). Copy number loss of chromosome 19 has been reported in 12.5% of neuroblastoma tumors (25, 26). No other chromosomal clustering of genes altered by DNMT3B7-expression was detected.

To identify genes that could contribute to the suppression of neuroblastoma cell growth in the presence of DNMT3B7, we focused on genes with the greatest fold changes by RNA-Seq that had CpG islands: ANKRD12 (22-fold), ASPM (15-fold), and EEA1 (14-fold; increased expression); and FOSB (46-fold), EGR1 (18-fold), and FOS (11-fold; decreased expression). In addition, we performed a GeneGo pathway analysis of all of the genes with at least a five-fold average change in expression with at least 150 reads per cell (Supplementary Table S2), which identified an additional five genes of interest: three genes that encode members of the AP1 complex that were underexpressed in the DNMT3B7-expressing cell lines FOSB (45-fold) and FOS (11-fold), both identified above, and JUNB (6-fold); genes important in neuronal function and tumorigenesis: KIF20B (11-fold), ROCK1 (6-fold), APC (5-fold), and RXRB (6-fold), encoding a retinoic acid signaling coregulator that heterodimerizes with retinoic acid receptors to bind on target gene promoters at the retinoic acid response element, which is important for retinoic acid treatment of children with high-risk neuroblastoma (12). In total, we performed quantitative real-time RT-PCR of all twelve genes of interest (Fig. 5A and B).
To test if the differential expression of genes in the DNMT3B7-expressing LA1-55n cells was the result of an alternation in their DNA methylation patterns, we performed bisulfite sequencing of eight genes that had been validated by real-time RT-PCR. Six of these genes showed no detectable DNA methylation across the transcriptional start site. RXRB, a gene important in retinoic acid signaling, showed a decrease expression of two-fold in DNMT3B7-expressing cells by real-time RT-PCR, and the CpG island surrounding the transcriptional start site was hypermethylated in the presence of DNMT3B7 (Fig. 5C). Similarly, EEA1 expression was nine-fold higher by real-time RT-PCR, and the CpG island upstream of the transcriptional start site became completely hypomethylated in DNMT3B7-expressing cells (Fig. 5D). The changes in DNA methylation were validated by LC/MS (Supplementary Fig. S8). These data show that expression of DNMT3B7 in neuroblastoma cells leads changes in gene expression, some of which are correlated to altered CpG island methylation.

**The effects of ATRA and DNMT3B7 expression on growth inhibition are additive**

RNA-Seq of the DNMT3B7-expressing LA1-55n cells revealed decreased expression of genes encoding components of the AP1 complex, FOSB (45-fold), FOS (11-fold), and JUNB (6-fold). In addition to acting as a transcription factor, AP1 can also antagonize the activity of retinoic acid receptors (27). We therefore hypothesized that, with reduced levels of AP1, retinoic acid receptors would be more active and could drive DNMT3B7-expressing cells toward differentiation. To test if retinoic acid treatment could augment the ability of DNMT3B7 to induce differentiation of neuroblastoma cells, we induced DNMT3B7 expression in the LA1-55n cells for 2 weeks to maximize DNMT3B7 expression (Fig. 2B). After 2 weeks of induction, both DNMT3B7-expressing control cells. Taken together, these results suggest a model in which the expression of DNMT3B7 induces neuroblastoma cells to differentiate, which can be promoted further by additional treatment by ATRA.

**Discussion**

DNMT3B catalyzes de novo methylation of DNA sequences, and high levels of this enzyme have been detected in cancer (36). In addition to full-length DNMT3B, more than 40 transcripts have been detected in common adult malignant tumors (18, 37). Many of the encoded proteins lack either the DNA binding or catalytic domains, and some have been shown to modify the pattern of DNA methylation and gene expression.
In this study, we found DNMT3B7 transcripts in all of the ganglioneuromas tested and at very high levels in all of the ganglioneuroblastomas, leading us to the hypothesis that DNMT3B7 could induce differentiation and lead to a more benign phenotype, thereby modifying tumor phenotype in pediatric neuroblastoma.

To test this hypothesis, we forced DNMT3B7 expression in neuroblastoma cells using retroviral vectors to drive either constitutive or inducible expression and evaluated its effects on DNA methylation, tumor growth, and angiogenesis. We observed growth inhibition of aggressive neuroblastoma cell lines and xenograft tumors with slowed tumor growth and suppression of angiogenesis. Although aberrant hypermethylation of specific tumor suppressor genes and a CpG-methylator phenotype have been associated with clinically aggressive neuroblastomas (37), cancer cells are globally hypomethylated compared to normal cells. Thus, the increase in global DNA methylation observed in neuroblastoma cells with forced DNMT3B7 expression and decreased tumor growth are consistent with a non-malignant phenotype.

Previously, we have demonstrated altered DNA methylation and gene expression in 293 cells overexpressing DNMT3B7 (18). In addition, a re-distribution of DNA methylation was observed in DNMT3B7 transgenic mice (21). Forced expression of other DNMT3B isoforms containing the catalytic domain (DNMT3B1-4) have been shown to induce hypermethylation of repetitive elements (38). The truncated DNMT3B variants that lack DNA binding or catalytic domains are likely to have altered function compared with DNMT3B, and may actually stimulate the activity.

Figure 5. RNA-Seq validation and CpG island methylation. A, validation of overexpression of six genes in DNMT3B7-expressing cells by real-time RT-PCR. B, validation of underexpression of six genes in DNMT3B7-expressing cells by real-time RT-PCR. C, bisulfite sequencing of RXRB, a gene downregulated in DNMT3B7-expressing cells. D, bisulfite sequencing of EEA1, a gene upregulated in DNMT3B7-expressing cells.
of other DNMTs with which it heterodimerizes (39), leading to hypermethylation.

GeneGo pathway analysis of the RNA-Seq data of the DNMT3B7-expressing LA1-55n cells identified several genes that may be important in neuroblastoma pathogenesis: KIF20B, ROCK1, ROCK2, APC and genes encoding members of the AP1 complex: FOSB, FOS, and JUNB. Increased expression of KIF1A has been found to correlate with high-risk neuroblastoma tumors (40).

ROCK1 (six-fold) and ROCK2 (six-fold) were both underexpressed in DNMT3B7-expressing cells, and activated RhoA and its downstream effector ROCK have been shown to be negative regulators of growth cone motility (41).

Adenomatous polyposis coli (APC) is a tumor suppressor gene, highly expressed in the developing and adult nervous system, which is involved in neurite formation (42, 43), and apoptosis of neural crest cells (44).

Additionally, GeneGo pathway analysis of the RNA-Seq data revealed decreased expression of genes encoding the components of the AP1 complex, which can antagonize the activity of retinoic acid receptors through protein-protein interaction without requiring DNA binding (27). We therefore hypothesized that, with reduced levels of AP1, retinoic acid receptors would be more active in the DNMT3B7-expressing cells, allowing augmentation of differentiation by retinoid treatment. Indicative of increased differentiation, DNMT3B7-expressing LA1-55n cells had higher expression of GFRA1 compared to vector controls following ATRA treatment. Similarly, the expression of DLK1 was decreased in both DNMT3B7-expressing LA1-55n cell lines following ATRA treatment (Fig. 6B). It has been shown previously that ATRA treatment of another MYCN amplified neuroblastoma cell line (SK-N-BE) leads to promoter hypomethylation (14), although we did not observe any significant global changes in DNA methylation after ATRA treatment (Supplementary Fig. S9). In the future, screening neuroblastoma tumors for DNMT3B7-expression may indicate particular patients for whom ATRA treatment might be particularly effective. DNMT3B exists in a protein complex with the mitotic chromatin condensation components, including HDAC1 and SIN3A (45). When unbound, retinoic acid receptors bind NCO2, SIN3A, and HDACs, leading to target promoter repression, which can be disassociated upon ATRA binding (46, 47).

Altered histone modifications have also been found in neuroblastoma tumors and have correlated to tumor aggressiveness. Elevated levels of EZH2, the enzymatically active component of the Polycomb Repressor Complex 2 and H3K27me3 marks have been identified at the promoters of tumor suppressors CASZ1, RUNXI, NGFR (p75), and NTRK1 (TrkA) in undifferentiated, poor prognosis neuroblastoma (48). Additionally, following retinoic acid induced differentiation of neuroblastoma cells, EZH2 expression is decreased, consistent with decreased binding of EZH2 to retinoic acid inducible target genes (49). Retinoic acid treatment therefore, leads to a reduction in repressive histone marks in addition to the changes in DNA methylation described above. Therefore, future work may focus on testing whether histone modifications are altered in this system.
Our findings suggest that forced expression of DNMT3B7 in neuroblastoma cells is able to drive genome-wide DNA methylation. This is similar to the effects seen in lymphomas that arise in Eμ-Myc/DNMT3B transgenic mice (21). However, in neuroblastoma, the expression of DNMT3B7 is anti-tumorigenic (Fig. 3), whereas DNMT3B expression in Eμ-Myc lymphomas accelerates tumorigenesis. There are many possible explanations for this: The observations are made in different species and cell types. In addition, there are no other aberrant DNMT3B transcripts present in the DNMT3B-Eμ-Myc lymphomas, whereas the human LA1-55n neuroblastoma cell expresses multiple DNMT3B transcripts. Truncated DNMT3B proteins may fine-tune the DNA methylation machinery within a cell dependent upon the other active DNMTs. These studies provide insight into understanding the molecular basis for the altered distribution of DNA methylation seen in virtually all human cancers. We hope that our discoveries will also provide a basis for novel diagnostic, prognostic, and therapeutic strategies that will be applicable to neuroblastoma and other types of tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: K.R. Ostler, Q. Yang, S.L. Cohn, L.A. Godley

Acknowledgments
We thank the Children’s Oncology Group (COG) Neuroblastoma Biology Committee for approving this study and providing the neuroblastoma cDNA samples, and Lisa J. Guerrero and Beau Blumenschein for their contribution to this work.

Grant Support
This work was supported by funding from Children’s Neuroblastoma Cancer Foundation (QY), and Comer Kids’ Classic Grant (QY), NIH grants U10 CA098413 (AN), U10 CA098543 (AN), The Neuroblastoma Children’s Cancer Society (SLC), Little Heroes Cancer Research Fund (SLC), Alex’s Lemonade Stand (SLC), Kimmel Scholars Program (LAG), American Cancer Society grant 08-02 (LAG), and NIH CA129831 (SLC).

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.

Received March 8, 2012; revised July 10, 2012; accepted July 11, 2012; published OnlineFirst July 18, 2012.

References

www.aacrjournals.org
Cancer Res; 72(18) September 15, 2012
OF9

Suppressed Neuroblastoma Growth
DNMT3B7 Suppresses Neuroblastoma Growth
OF9

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.R. Ostler, L. Zhang, A. Vasanthakumar, Y. Tian, S.L. Cohn, J.G. DeMaio, S. Gu, A. Gill, R. Peddinti, B.T. Lahn, S.L. Cohn, L.A. Godley
Analysis and interpretation of data (e.g., statistical analysis, bioinformatics, computational analysis): K.R. Ostler, Q. Yang, T.J. Looney, A. Vasanthakumar, Y. Tian, M. Koehberginsky, S.L. Raimondi, H.R. Salwen, S. Gu, A. Chlenski, A. Naranjo, B.T. Lahn, S.L. Cohn, L.A. Godley
Writing, review, and/or revision of the manuscript: K.R. Ostler, Q. Yang, L. Zhang, S.L. Raimondi, J.G. DeMaio, H.R. Salwen, S. Gu, A. Chlenski, A. Naranjo, R. Peddinti, S.L. Cohn, L.A. Godley
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Q. Yang, L. Zhang, Y. Tian, H.R. Salwen, S.L. Cohn
Study supervision: Q. Yang, S.L. Cohn, L.A. Godley

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: K.R. Ostler, Q. Yang, S.L. Cohn, L.A. Godley

Acknowledgments
We thank the Children’s Oncology Group (COG) Neuroblastoma Biology Committee for approving this study and providing the neuroblastoma cDNA samples, and Lisa J. Guerrero and Beau Blumenschein for their contribution to this work.

Grant Support
This work was supported by funding from Children’s Neuroblastoma Cancer Foundation (QY), and Comer Kids’ Classic Grant (QY), NIH grants U10 CA098413 (AN), U10 CA098543 (AN), The Neuroblastoma Children’s Cancer Society (SLC), Little Heroes Cancer Research Fund (SLC), Alex’s Lemonade Stand (SLC), Kimmel Scholars Program (LAG), American Cancer Society grant 08-02 (LAG), and NIH CA129831 (SLC).

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.

Received March 8, 2012; revised July 10, 2012; accepted July 11, 2012; published OnlineFirst July 18, 2012.


Truncated DNMT3B Isoform DNMT3B7 Suppresses Growth, Induces Differentiation, and Alters DNA Methylation in Human Neuroblastoma

Kelly R. Ostler, Qiwei Yang, Timothy J. Looney, et al.

Cancer Res  Published OnlineFirst July 18, 2012.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-12-0886

Supplementary Material  Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2012/07/18/0008-5472.CAN-12-0886.DC1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.