Identification of T-cadherin as a Novel Target of DNA Methyltransferase 3B and Its Role in the Suppression of Nerve Growth Factor-mediated Neurite Outgrowth in PC12 Cells

Shoumei Bai, Kalpana Ghoshal, and Samson T. Jacob

From the Department of Molecular and Cellular Biochemistry, College of Medicine, Ohio State University, Columbus, Ohio 43210

Previously we showed that DNA methyltransferase 3b (Dnmt3b) is required for nerve growth factor (NGF)-induced differentiation of PC12 cells to neuronal phenotype. These results demonstrate a novel role of T-Cad in the NGF-mediated neurite outgrowth of PC12 cells induced by NGF. Immunofluorescence studies suggest that T-Cad is localized at cell-cell contact region in undifferentiated cells. Interestingly, immunoprecipitation studies showed that T-Cad interacted with the catalytic domain of Dnmt3a, which is consistent with the finding that Dnmt3a is a repressor of T-Cad. Moreover, the expression of T-Cad was decreased in cells depleted of Dnmt3b by antisense oligonucleotides. These data suggest that T-Cad is a target of Dnmt3b and is transcriptionally repressed by Dnmt3b in PC12 cells.

This article has been withdrawn by the authors. In June 2017, the Journal raised questions concerning Figs. 2B, 3B, and 5A. The original data and originally submitted figures were not available for evaluation. Regarding Fig. 2B, the authors were able to locate a repeated experiment performed at the time of the original work, which they state confirm the results. Regarding Figs. 3B and 5A, the authors are not convinced that any duplication occurred, and they were able to provide to the Journal data from multiple partial repeat experiments performed at the time of the original work, which they state confirm the results. The authors offered to publish substitute figures based on the repeated experiments and, alternatively, offered to repeat the experiments. However, the Journal declined both of these offers, a decision with which the authors disagree. The authors stand by the reproducibility of the experimental data and the conclusions of the paper. The paper, with confirmatory data supporting the results, can be obtained by contacting the authors.
ture condition, where T-Cad blocks neurite extension of spinal motor neurons both as a substratum and as a soluble recombiant protein (20). A high T-Cad level is also found in cardiac and vascular tissues where aryl hydrocarbon receptor ligands can repress its expression. T-Cad is co-segregated with signaling molecules such as G-protein and SRC family kinase, which implicates its role as an intracellular signaling molecule (25). Interestingly, T-Cad can also function as a receptor for low density lipoprotein and adiponectin/Acrp30 (26, 27). T-Cad is down-regulated by growth factors such as IGF, EGF, and bDGF in smooth muscle cells (28). The molecular mechanism for its down-regulation by these growth factors has not been elucidated. The identification of T-Cad as one of the target genes of DNA methyltransferase 3b coupled with increased expression of this DNA methyltransferase during differentiation of PC12 cells provided an important experimental system to study the function of T-Cad in the differentiation process. The present study addresses the mechanism by which Dnmt3b suppresses T-Cad expression and the role of T-Cad in NGF-induced differentiation of PC12 cells.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Trichostatin A (TSA) was from Sigma. NGF was from Roche Applied Science. Anti Dnmt3b antibody was raised in our laboratory as described previously (29). Anti-T-Cad and anti-HDAC2 antibodies were from Santa Cruz Biotechnology. Anti-NGF was from Roche Applied Science. Anti Dnmt3b antibody was with NGF as described earlier (16).

**Cell Line and Tissue Culture**—PC12 cells were cultured and treated with NGF as described earlier (16).

**Construction of Plasmids and Generation of Stable Cell Lines**—T-Cad-FLAG (T-CadFLAG): T-Cad cDNA was ampliﬁed from lung cDNA library (primers 5'CGG GAT CCT GGC AAG ATG CA, and 5'-CGG AAT CCT GGT AAG ATG CA, and 5'-CGGATCCCAGACCTGACAATAAGCTGA as the reverse primer) or its isoschizomer MspI (methylation-insensitive) followed by PCR and input DNA were digested with either HpaII (methylation-sensitive) or its isoschizomer MspI (methylation-insensitive) followed by PCR. The PCR products were then ligated into pCMV-3’-FLAG (Sigma). To obtain T-Cad-FLAG, T-CadFLAG was transfected into PC12 cells and selected with G418 (500 µg/ml). Expression of T-Cad in stable cell lines was veriﬁed by Western blot analysis with anti-FLAG M2 antibody.

**T-Cad Promoter-Luciferase Reporter Gene (pT-CadGL2)—**T-Cad promoter was PCR-ampliﬁed from PC12 cell genomic DNA with primers 5’CGG GGT ACC ACC ATG CTG GGC AAC ATG CTG T and 5’-CCG CTC GAG TGC GGC TCA CAT TCC CTA CCTG and cloned into the KpnI and Xhol site of pGL2 basic plasmid (Promega).

**Dnmt3b-ΔPWWP—**PWWP domain deletion mutant of Dnmt3b was generated by PCR as described earlier (16) with the primers 5’-CGG GGT ACC ACC ATG CTG GGC AAC ATG CTG T and 5’-CCG CTC GAG TGC GGC TCA CAT TCC CTA CCTG and cloned into the KpnI and XhoI site of pGL2 basic plasmid (Promega).

**Dnmt3b Represses T-cadherin Expression**—Cells were treated with bisulfite reagent as described (31). T-Cad CpG island (CGI) located at proximal promoter and exon 1 was ampliﬁed by nested PCR with primer set #1 (proximal promoter) (5'-GGT TGG TTG TTA GGA GTA AAA and AAA ACC AAC CCTT TAA AAA AAA) and set #2 (exon) (5'-TTT TTG GGA GTT TGG TGG G and 5'-CTC ACA TTC ACC ATT AAC). The PCR products were puriﬁed and digested with BstUI or TaqI to determine the methylation status. Tsp509I was used to verify the completion of bisulfite conversion. For bisulfite sequencing, the PCR products were cloned into TOPO-TA vector (Invitrogen), and puriﬁed plasmid DNA from individual clones from each set was sequenced.

**Bisulfite Genomic Sequencing—**ChIP assay was performed as described earlier (22) and cells were cross-linked with 1% formaldehyde. Chromatin was sonicated by sonicating the cell lysate that was sonicated for 10 min on ice chamber. Chromatin for Dnmt3b or protein G-agarose A or Hdac2 associated protein-DNA complex was pulled down with anti-Dnmt3b or anti-Hdac2 antibody, respectively, and immunoprecipitated with the same antibody to minimize nonspeciﬁc precipitation. The PCR assay buffer and subjected to one more round of in immunoprecipitation with the same antibody to minimize nonspeciﬁc pull-down. To identify Dnmt3b target genes the DNA fragments pull-down by Dnmt3b were subjected to end filling/repairing with Klenow and subsequently cloned into the 5′smal site of pBlueScript-SK + to allow blue white color selection. Individual clones were identiﬁed by automated sequencing. The identity of the potential genes was established with gene analyzing tools and gene databases (BLAST: www.ncbi.nlm.nih.gov/BLAST; BLAT: genome.ucsc.edu/cgi-bin/hgBlat; SOURCE: genome-www5.stanford.edu/cgi-bin/source/; Uniprot: www.expasy.uniprot.org/). The primers used to amplify the T-Cad promoter were 5′-AGG GAG CGT TAT GAA GGA ATCC and 5′-GAG GAC ACC AAG TATA TGG TAC AAG TAAA and the primers used for GAPDH promoter were 5′-TCA CGG GGT GTT GTG GTCC TGC TCC CGT GTT and 5′-TTT CGC TGG GTG TGT GTG TGT GC TGC TGG TGT TGT GC for amplification of T-Cad promoter. The PCR products were cloned into five individual clones from each set were sequenced with gene analyzing tools and gene databases (BLAST: www.ncbi.nlm.nih.gov/BLAST; BLAT: genome.ucsc.edu/cgi-bin/hgBlat; SOURCE: genome-www5.stanford.edu/cgi-bin/source/; Uniprot: www.expasy.uniprot.org/).

**In Vitro DNA Methylation—**The protocol used for the site-speciﬁc methylation of T-Cad was as described (33).

**Transient Transfection and Luciferase Assay—**PC12 cells were plated at a density of 5 × 10^5/12-well plate, transfected with T-Cad promoter (pT-CadGL2 along with Dnmt3b or empty vector using Lipofectamine 2000TM (Invitrogen). Cells were harvested 48 h post-transfection followed by the luciferase reporter assay in the cell lysate (29). Transfection of Dnmt3b siRNA was performed as described (16).
Dnmt3b Represses T-cadherin Expression

Indirect Immunofluorescence Assay—PC12 cells were plated in Lab-Tek chamber and fixed with 4% paraformaldehyde, permeabilized, and incubated in blocking buffer (1% bovine serum albumin, 10% goat serum) for 1 h, followed by primary antibody overnight at 4 °C. Subsequently, the cells were incubated with TRITC-conjugated secondary antibody (for M2 antibody), mounted in 4',6-diamidino-2-phenylindole containing mounting medium, and visualized under immunofluorescence microscopy.

RESULTS

T-Cad Is a Dnmt3b Target Gene in PC12 Cells—To explore the molecular mechanism of Dnmt3b-mediated differentiation of PC12 cells, we identified the potential targets in these cells. For this purpose, the Dnmt3b-DNA complex was immunoprecipitated with Dnmt3b antibody and the purified DNA was cloned into pBSK+. Approximately 120 clones were sequenced. The identity of the potential target genes was established by data base analysis. Based on their known or postulated function, Dnmt3b target genes were classified into five categories, namely, cell matrix/transmembrane proteins, proteins specific to brain/neuron, signaling transduction, cell cycle progression, and transcriptional regulation (see supplemental Table S1). Approximately 10% of the pulled down sequences were ALU repeats, single repeats, or long terminal repeats (not listed).

We selected one of the target genes, T-Cad, for further study based on the important role of the encoded protein in cell-cell adhesion, cell signaling, embryogenesis, and in motor neuron axon growth (20, 12). The association of Dnmt3b with T-Cad promoter in PC12 cells was verified by chromatin immunoprecipitation followed by PCR analysis. T-Cad promoter was specifically amplified from immunoprecipitated with Dnmt3b antibody but not from preimmune serum. The result showed that Dnmt3b protein is elevated in Dnmt3b antisense (Dnmt3bAS) cells. Whole cell extract was prepared with Dnmt3b and Ku-70 antibodies. Western blot analysis demonstrated significant depletion of Dnmt3b in cells expressing antisense 3b (Fig. 1B, lower panel). T-Cad mRNA was also up-regulated in cells depleted of Dnmt3b using siRNA (Fig. 1C, top panel). The level of T-Cad 48 h post-transfection increased proportionately (~2-fold) with the concentration of Dnmt3b siRNA used, whereas the same amount of scrambled siRNA did not have any significant effect. The increase in T-Cad mRNA level after 72 h was less pronounced (Fig. 1C, top panel), which is consistent with the increase in Dnmt3b mRNA (Fig. 1C, middle panel) probably due to decrease in intracellular siRNA level with time. No significant alteration in Dnmt3b protein level was detected in scrambled siRNA transfected cells compared with that of the parental cells whereas its level was reduced by 75% in Dnmt3b-siRNA transfected cells (Fig. 1C, bottom panel). Western blot analysis showed that T-Cad protein level significantly increased upon Dnmt3b depletion in PC12 cells (Fig. 1D) that correlated with increase in its mRNA level (Fig. 1, B and C). These results demonstrate that Dnmt3b negatively regulates T-Cad expression. Dnmt3b level was not altered in vector-transfected cells compared with that of parental cells (data not shown).

T-cadherin CGI Is Partially Methylated in PC12 Cells, Which Is Not Significantly Altered upon Dnmt3b Depletion—To identify the underlying mechanism of Dnmt3b-mediated down-regulation of T-Cad expression, we first investigated whether Dnmt3b regulates the methylation status of T-Cad promoter in PC12 cells. To address this issue, we performed COBRA (combined bisulfite-restriction analysis) of T-Cad promoter CGI in the vector-transfected and Dnmt3b depleted PC12 cells. Bisulfite treatment of DNA converts unmethylated cytosines to uracils, whereas methylated cytosines remain unaffected. Upon subsequent PCR, uracils and methylcytosines are amplified as thymine and cytosines, respectively. The amplified PCR product from only methylated DNA could be digested by enzymes such as TaqI (TCGA) or BstUI (C GCCG) due to retention of cytosine. The PCR products obtained from two regions of the T-Cad CGI spanning the proximal promoter (~220 to ~38) and first exon (~38 to +96) (Fig. 2A) were digested with these enzymes. The results showed that the promoter was partially digested with these enzymes demonstrating partial methylation (Fig. 2B, left and middle panels). The methylation of proximal promoter was significantly less (~20%) than that of exon 1 (~40%) (Fig. 2B, left and middle panels). Complete digestion of the PCR products with Tsp509I confirmed complete bisulfite conversion (Fig. 2B, right panel). It is noteworthy that the
FIGURE 2. Methylation status of the CGI located on T-Cad promoter and exon 1 is not significantly altered upon Dnmt3b depletion. A, schematic diagram showing the distribution of CpGs on T-Cad (T-Cad) promoter and exon 1 CGI. The bar represents CpG dinucleotides. The diamond, oval, and open arrows represent BstUI, TaqI, and Tsp509I sites, respectively, in bisulfite-converted T-Cad DNA. +1 indicates the transcription start site. Arrows with solid and broken lines represent the primers used spanning the proximal promoter and exon 1, respectively. B, COBRA of T-Cad CGI. Genomic DNA isolated from Dnmt3b antisense cells (Dnmt3bAS) and vector-transfected control cells was treated with bisulfite reagent, amplified with T-Cad-specific primers with no CpG bias, followed by digestion with BstUI, TaqI, or Tsp509I. Products were separated on an agarose gel, visualized after staining with ethidium bromide, and quantified with Kodak imaging software. Left panel, PCR products spanning proximal promoter of T-Cad were digested with BstUI, Middle panel, TaqI digestion of the PCR product spanning exon 1. Right panel, Tsp509I digestion of T-Cad promoter and exon 1. C, bisulfite genomic sequencing analysis of T-Cad promoter in Dnmt3b-depleted cells (Dnmt3bAS) and vector-transfected PC12 cells (vector). PCR products from B.1 and B.2 were cloned with TA-vector and sequenced. The open square and filled square represent unmethylated and methylated cytosine, respectively. The positions of the CpGs are labeled on top of each box.

Partial methylation of T-Cad promoter raised the question whether Dnmt3b is preferentially associated with the methylated promoter. To address this issue ChIP-CHOP assay was performed. DNA pulled down with preimmune sera or Dnmt3b antibodies was either undigested or digested with HpaII or MspI. The extent of digestion of the PCR product obtained from Dnmt3bAS cells with BstUI (Fig. 2B, left panel) or TaqI (Fig. 2B, middle panel) is comparable with that of vector-transfected cells. This suggests a similar methylation profile of individual CpG bases in the T-Cad, bisulfite genomic sequencing analysis, and bisulfite genomic sequence analysis revealed sparsely methylated T-Cad promoter region and comparatively dense methylation of CpGs in the proximal promoter region and especially dense methylation of CpGs in the exon 1 in both Dnmt3b-depleted cells (Fig. 2C), which corroborated the COBRA data. These results suggest that T-Cad CGI is not heavily methylated in PC12 cells, which is maintained in the absence or presence of Dnmt1 and/or Dnmt3a.

Mock-methylated or HpaII-methylated T-Cad promoter was co-transfected with the wild type or different mutants of Dnmt3b (Fig. 4A). The T-Cad promoter-driven luciferase activity was significantly impeded (∼50%) when the promoter was methylated at HpaII sites (Fig. 4A, lanes 2 and 5). The extent of inhibition of Dnmt3b on both promoters was comparable (55 and 58% on mock-methylated and methylated promoters, respectively). It is noteworthy that the inhibitory

FIGURE 3. Dnmt3b is associated with both unmethylated and methylated T-Cad promoter as revealed by ChIP-CHOP assay. A, schematic depiction of ChIP-CHOP assay. Formaldehyde cross-linked DNA (Input) or Dnmt3b antibody pulled down DNA was digested with methylation-sensitive restriction enzyme HpaII (H) or methylation-insensitive enzyme MspI (M) or mock-digested (−) followed by PCR analysis with T-Cad or GAPDH promoter-specific primers. B, ethidium bromide staining of the amplified product separated on an agarose gel. C, quantitative analysis of the association of Dnmt3b with the T-Cad promoters as assessed by the ratio of HpaII-resistant product to input PCR product.
Dnmt3b Represses T-cadherin Expression

Effect of the catalytic site (CS) mutant of Dnmt3b on both T-Cad promoters was similar (~60%) to that of the wild type protein (lanes 3 and 6). The level of the ectopic wild type and CS mutant Dnmt3b was also comparable (Fig. 4B, lower panel). These results indicate that Dnmt3b suppresses T-Cad promoter activity irrespective of its methylation status and its catalytic activity is not required for this process. Furthermore, the extent of repression of T-Cad promoter activity increased with increasing amount of transfected Dnmt3b expression vector (Fig. 4C).

To determine whether the transfected T-Cad promoter is methylated de novo upon ectopic expression of Dnmt3b, the reporter plasmid (pT-cadGL2) was isolated from PC12 cells co-transfected with Dnmt3b expression vector or the corresponding empty vector. The plasmid DNA was subjected to digestion with methylation sensitive restriction enzyme HpaII, AciI, or methylation-insensitive MspI, followed by PCR analysis with T-Cad-specific forward primer and pGL2 specific reverse primer. Lack of amplification of the T-Cad promoter fragment from HpaII- or AciI-digested plasmid DNA showed that the promoter was not methylated when co-expressed with Dnmt3b or empty vector (Fig. 4D). These results further prove that the Dnmt3b-mediated suppression of T-Cad promoter activity does not involve promoter methylation. Furthermore, inability of 5-azacytidine to increase basal expression of the endogenous and transfected T-Cad promoter (data not shown) supports the notion that methylation does not regulate its expression in PC12 cells, although its promoter is sparsely methylated (Fig. 2C).
were either left untreated or treated with 3 nM TSA for an additional 18 h. Cell lysate was transfected with pT-cadGL2 along with Dnmt3b or vector (pCMV) and after 24 h cells or treated with 3 nM TSA for 18 h was subjected to real-time RT-PCR analysis. The mRNA level was normalized to the cell number from each transfection.

Apart from the catalytic domain, Dnmt3b harbors a relatively large N-terminal region that encompasses PWWP and ATRX domains (35–44). Although PWWP domain of Dnmt3b exhibits DNA methylation activity (35), Dnmt3b can still binds to DNA in its absence, involved in protein-protein interaction (37, 38) and homologous to the ATRX gene, a member of the SWI/SNF family of chromatin remodeling proteins (39). ATRX domain can recruit histone deacetylases (Hdacs) and other transcriptional co-repressors to inhibit chromatin remodeling proteins (39). ATRX domain can recruit histone deacetylases (Hdacs) and other transcriptional co-repressors to inhibit chromatin remodeling proteins (39). ATRX or PWWP mutants. The inhibitory effect of Dnmt3b on the promoter activity was co-precipitated upon deletion of PWWP or ATRX domain (Fig. 4, lower panel). Deletion of mock-methylated promoter was 61 and 37%, respectively, versus 79% by the wild type; methylated promoter was 42 and 30%, respectively, versus 58% by the wild type). Western blot analysis demonstrated comparable expression of Dnmt3b variants (Fig. 4, B and E). The partial reversal of its inhibitory effect on T-Cad promoter upon deletion of these domains suggests that both PWWP and ATRX domain are essential for the repressor function of Dnmt3b on T-Cad promoter irrespective of its methylation status. These results were further confirmed by ectopic expression of the entire N-terminal deletion mutant of Dnmt3b (ΔN), where its inhibitory effect on T-Cad promoter was abolished (Fig. 4F). The inhibitory effect of Dnmt3b on T-Cad promoter varied slightly between experiments (Fig. 4, B and E), which was possibly due to differential expression of Dnmt3b.

Hdac2 Is Associated with T-Cad Promoter, and the Inhibitory Activity of Dnmt3b on the Promoter Can Be Relieved by Treatment with TSA, a Hdac Inhibitor—The involvement of ATRX domain of Dnmt3b in the repression of T-Cad suggested to us the potential involvement of Hdac as a co-repressor. Our earlier study demonstrated Dnmt3b physically and functionally interact with Hdac2 in PC12 cells differentiated by NGF (16). To investigate whether Hdac2 plays a role in the regulation of the expression of T-Cad, we performed ChIP assay with Hdac2 specific antibody. Indeed, Hdac2 was associated with T-Cad promoter (Fig. 5A, upper panel, lane 3). No PCR product was detected when protein G-agarose beads alone were used for the assay (lane 2). Lack of amplification of PI3K promoter further showed the specificity of this association (Fig. 5A, lower panel). Next, we measured T-Cad mRNA level in cells treated with TSA. The result showed dramatic increase (~25-fold) in T-Cad mRNA upon treatment with TSA (Fig. 5B). Similarly, TSA treatment also activated the transfected promoter by 4.5-fold (Fig. 5C) suggesting that Hdacs also regulate the activity of the promoter. Significantly less activation of the ectopic promoter compared with that of the mRNA level is probably due to the availability of a small fraction of the transfected promoter in the nucleosomal structure. Alternatively, the transfected promoter (~1.6 kb) cannot execute full function due to lack of some regulatory elements. However, the results demonstrate that both endogenous and the transfected promoters are regulated by Hdacs. The next obvious question was whether Dnmt3b repressed T-Cad by recruiting Hdac. A 7.6-fold increase in T-Cad promoter activity in Dnmt3b transfected cells upon TSA treatment showed that suppression of histone deacetylase activity could overcome the inhibitory activity of Dnmt3b on the promoter (Fig. 5C). This result suggests that the recruitment of Hdacs, probably Hdac2, by Dnmt3b is essential to repress the T-Cad promoter.

T-Cad Inhibits NGF-induced Neurite Outgrowth in PC12 Cells—To investigate the potential role of T-Cad in NGF-induced PC12 cell differentiation, stable cell lines constitutively expressing FLAG-tagged T-Cad (T-CadFLAG) were generated. Two cell lines expressing low (T-Cad1) and high (T-Cad2) levels of T-Cad (Fig. 6A) were used for further studies. First, the neuronal differentiation was examined in these cells treated with NGF for up to 6 days and compared with that of vector-transfected (control) cells. Neurite outgrowth was visible within 24 to 48 h of NGF treatment in control cells (Fig. 6A). In contrast, both...
DISCUSSION

The present study explored the role of T-Cad in neuronal differentiation using NGF-mediated neurite outgrowth in PC12 cells as the model system. Specific increase in Dnmt3b level, one of the three functional DNA methyltransferases, reported in our earlier study (16) and the present observation that T-Cad is one of the Dnmt3b target genes prompted us to explore the functional significance of this finding. Interestingly, Dnmt3b suppressed T-Cad expression with no significant effect on its promoter methylation. Furthermore, depletion of Dnmt3b from PC12 cells enhanced its expression, which correlated with continued proliferation of the cells in the presence of NGF (16). This result reinforces the notion that T-Cad functions as a negative signal in neuronal differentiation. In this context, it is noteworthy that T-Cad functions in initiating proliferation in vascular cells and that it may facilitate progression of proliferative vascular disorders (46). There are also indications that human T-Cad is involved in lung metastasis of osteosarcoma (47). The abundant expression of T-Cad in undifferentiated or metastatic cancer cells is consistent with the lack of or markedly reduced expression of T-Cad in differentiated sympathetic neurons in vivo (20) and in differentiated PC12 cells observed in the present study.

The molecular mechanism underlying T-Cad-mediated inhibition of neurite outgrowth remains to be resolved. It appears that it can function as a negative regulator of axon growth. It is expressed in the region devoid of extending growing motor axons during chick embryo hind limb projection, which implies that T-Cad functions as an inhibitory factor for motor axon growth (48). The present study showed re-distribution of T-Cad during NGF-induced differentiation of PC12 cells as shown by its strong staining in restricted membrane region and growing axon processes after NGF treatment in contrast to dense staining at cell-cell contact in undifferentiated PC12 cells (Fig. 6, B and C). Thus, T-Cad1 cells (low T-Cad level) responded poorly to NGF treatment (Fig. 6, B and C). A significant decrease of the clusters in T-Cad-expressing cells, and this reduction was inversely correlated with the level of T-Cad (Fig. 6, B and C). Therefore, the redistribution of T-Cad to growth cone in response to NGF might have a role in its inhibition of neurite outgrowth in PC12 cells.

T-Cad Is Redistributed from Cell-Cell Contact Region to Neurite Growth Cones in Differentiated Cells

As a predominantly an extracellular protein associated with the plasma membrane through GPI anchor. Because NGF-induced differentiation of PC12 cells causes profound changes on the plasma membrane that include membrane depolarization (42) and translocation or redistribution of membrane-conjugated signaling molecules such as TrkA (43), PKCα (44), and inositol polyphosphate 5-phosphatase (IPP) (45), we examined the localization of T-Cad after NGF treatment. T-Cad exhibited punctate staining at the plasma membrane and dense staining was observed at cell-cell contact points in untreated cells (Fig. 7, A and C), which indicated its localization at the cell-cell contact points. Strikingly, T-Cad was redistributed upon NGF treatment as demonstrated by its localization at neurite growth cones and growing axon processes (Fig. 7, D and F, G and I, and J and L). The re-distribution of T-Cad to growth cone in response to NGF might have a role in its inhibition of neurite outgrowth in PC12 cells.
cancer and has provided the impetus to explore the molecular mechanism of T-Cad signaling as well as regulation of its expression.

Acknowledgments—We thank Drs. Chih-Lin Hsieh for providing cDNAs for the wild type and catalytic domain mutant of Dnmt3b, En Li for Dnmt3b cDNA, and Sarmila Majmudar and Tanzeem Motiwala for critically reading the manuscript.

REFERENCES

1. Goll, M. G., and Bestor, T. H. (2005) Annu. Rev. Biochem. 74, 481–514
2. Hermann, A., Gowher, H., and Jeltsch, A. (2004) Cell. Mol. Life Sci. 61, 2571–2587
3. Li, E., Bestor, T. H., and Jaenisch, R. (1992) Cell 69, 915–926
4. Okano, M., Bell, D. W., Haber, D. A., and Li, E. (1999) Cell 99, 247–257
5. Datta, J., Majumder, S., Bai, S., Ghoshal, K., Kutay, H., Smith, D. S., CraBB, J. W., and Jacob, S. T. (2005) Cancer Res. 65, 10891–10900
6. Fuks, F., Burgers, W. A., Brehm, A., Hughes-Davies, L., and Kouzarides, T. (2000) Nat. Genet. 24, 88–91
7. Fuks, F., Hurd, P. J., Wolf, D., Nan, X., Bird, A. P., and Kouzarides, T. (2003) J. Cell Biol. 162, 539–546
8. Geiman, T. M., and Robertson, K. D. (2002) J. Cell. Biochem. 82, 380–392
9. Motiwala, T., Ghoshal, K., Datta, J., Majumder, S., Weichenhan, D., Wu, Y. Z., Holman, K., James, S. J., Jacob, S. T., and Plass, C. (2003) Oncogene 22, 6319–6331
10. Herman, J. G., and Baylin, S. B. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9815–9818
11. Baylin, S. B., and Herman, J. G. (2001) Annu. Rev. Biochem. 70, 267–300
12. Herman, J. G., and Baylin, S. B. (2000) Cancer Res. 60, 4927–4934
13. Ranscht, B., and Bronner-Fraser, M. (1991) J. Cell. Biol. 114, 183–187
14. Geiman, T. M., and Robertson, K. D. (2002) J. Cell Biol. 156, 217–224
15. Cameron, E. E., Bachman, K. E., Myohanen, S., Herman, J. G., and Baylin, S. B. (1999) Cancer Res. 59, 8328–8333
16. Bai, S., Ghoshal, K., Datta, J., Majumder, S., Kutay, H., Smith, D. S., CraBB, J. W., and Jacob, S. T. (2005) Cancer Res. 65, 10891–10900
17. Takeuchi, T., Liang, S. B., and Ohtsuki, Y. (2002)
18. Ranscht, B., and Bronner-Fraser, M. (1991) J. Cell. Biol. 114, 183–187
19. Takeuchi, T., Misaki, A., Liang, S. B., Tachibana, A., Hayashi, N., Sonobe, H., and Ohtsuki, Y. (2000) J. Neurosci. 20, 13611–13618
20. Takeuchi, T., Misaki, A., Chen, B. K., and Ohtsuki, Y. (1999) J. Cell Biol. 145, 1003–1010
21. Takeuchi, T., Misaki, A., Chen, B. K., and Ohtsuki, Y. (1999) J. Cell Biol. 145, 1003–1010
22. Takeuchi, T., Misaki, A., Chen, B. K., and Ohtsuki, Y. (1999) J. Cell Biol. 145, 1003–1010
23. Sato, M., Mori, Y., Sakurada, A., Fujimura, S., and Horii, A. (1998) J. Cell Sci. 111, 2571–2587
24. Widschwendter, A., Ivarsson, L., Blassnig, A., Muller, H. M., Fiegl, H., Wiedemair, A., and Jacob, S. T. (2005) Cancer Res. 65, 10891–10900
25. Datta, J., Majumder, S., Bai, S., Ghoshal, K., Kutay, H., Smith, D. S., CraBB, J. W., and Jacob, S. T. (2005) Cancer Res. 65, 10891–10900
26. Hug, C., Wang, J., Ahmad, N. S., Bogan, J. S., Tsao, T. S., and Lodish, H. F. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10308–10313
27. Tkachuk, V. A., Bochkov, V. N., Philippova, M. P., Stambolsky, D. V., Kuzmenko, E. S., Sidorova, M. V., Molokoevod, A. S., Spirov, V. G., and Resink, T. J. (1998) FEBS Lett. 429, 207–210
28. Kuzmenko, Y. S., Kern, F., Bochkov, V. N., Tkachuk, V. A., and Resink, T. J. (1998) FEBS Lett. 429, 207–210
29. Majumder, S., Ghoshal, K., Datta, J., Bai, S., Dong, X., Quan, N., Plass, C., and Jacob, S. T. (2002) J. Biol. Chem. 277, 16048–16058
30. Chornocka, P., and Ranscht, B. (1994) J. Neurosci. 14, 7331–7346
31. Takeuchi, T., Misaki, A., Sonobe, H., Liang, S. B., and Ohtsuki, Y. (2000) J. Neurosci. 20, 13611–13618
32. Deplus, R., Brenner, C., Buffe, A., Schneeburger, P., Raux, E., and Higgs, D. R. (2000) Nat. Genet. 25, 2536–2544
33. Camerino, E., Bachman, K. E., Myohanen, S., Herman, J. G., and Baylin, S. B. (1999) Cancer Res. 59, 8328–8333
34. Angst, B. D., Marcozzi, C., and Magee, A. I. (2001) J. Cell Biol. 153, 132–143
35. Deplus, R., Brenner, C., Buffe, A., Schneeburger, P., Raux, E., and Higgs, D. R. (2000) Nat. Genet. 25, 2536–2544
36. Datta, J., Majumder, S., Bai, S., Ghoshal, K., Kutay, H., Smith, D. S., CraBB, J. W., and Jacob, S. T. (2005) Cancer Res. 65, 10891–10900
37. Fuks, F., Burgers, W. A., Godin, N., Kasai, M., and Kouzarides, T. (2001) EMBO J. 20, 2536–2544
38. Shimazu, K., Takeda, K., Sugino, A., Sato, M., Liang, H., Liu, X. W., Nelson, P. G., and Guroff, G. (2005) J. Cell. Physiol. 202, 437–444
39. Takeuchi, T., Misaki, A., Sonobe, H., Liang, S. B., and Ohtsuki, Y. (2000) J. Neurosci. 20, 13611–13618
40. Gibbons, R. J., McDowell, T. L., Raman, S., O’Rourke, D. M., Garrick, D., Ayubh, H., and Higgs, D. R. (2000) Nat. Genet. 24, 368–371
41. Fuks, F., Burgers, W. A., Godin, N., Kasai, M., and Kouzarides, T. (2001) EMBO J. 20, 2536–2544
42. Reddy, E. C., and Baylin, S. B. (2000) Biochim. Biophys. Acta 1489, 207–214
43. Hibi, K., Koderia, Y., Ito, K., Akiyama, H., Fujimura, S., and Horii, A. (1999) J. Cell Sci. 112, 31–37
44. Marin-Vicente, C., Gomez-Fernandez, J. C., and Sarmila Majmudar, Tasneem Motiwala for critically reading the manuscript.
Identification of T-cadherin as a Novel Target of DNA Methyltransferase 3B and Its Role in the Suppression of Nerve Growth Factor-mediated Neurite Outgrowth in PC12 Cells

Shoumei Bai, Kalpana Ghoshal and Samson T. Jacob

J. Biol. Chem. 2006, 281:13604-13611.
doi: 10.1074/jbc.M513278200 originally published online March 14, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M513278200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2006/03/16/M513278200.DC1

This article cites 56 references, 15 of which can be accessed free at
http://www.jbc.org/content/281/19/13604.full.html#ref-list-1

WITHDRAWN
February 13, 2018