NIH Public Access
Author Manuscript
Biochem J. Author manuscript; available in PMC 2013 December 17.

Published in final edited form as:
Biochem J. 2012 September 1; 446(2): . doi:10.1042/BJ20120768.

Protein arginine methyltransferase 5 is essential for growth of lung cancer cells

Zhongping Gu[*,†], **Shen Gao**[*], **Fahao Zhang**[*], **Wang Zhiqiang**[‡], **Wencai Ma**[‡], **Richard E. Davis**[‡], and **Zhengxin Wang**[*]

[*]Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas

[‡]Department of Lymphoma/Myeloma, The University of Texas MD Anderson Cancer Center, Houston, Texas

[†]Department of Thoracic Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an, China

Abstract

Protein arginine methyltransferase 5 (PRMT5) is an enzyme that catalyzes transfer of methyl groups from S-adenosyl methionine to the arginine residues of histones or nonhistone proteins and is involved in a variety of cellular processes. Although it is highly expressed in some tumors, its direct role in cancer growth has not been fully investigated. In this study, in human lung tissue samples, we found that PRMT5 was highly expressed in lung cancer cells whereas its expression was not detectable in benign lung tissues. Silencing PRMT5 expression strongly inhibited proliferation of lung adenocarcinoma A549 cells in tissue culture, and silencing PRMT5 expression in A549 cells also abolished growth of lung A549 xenografts in mice. *In vitro* and *in vivo* studies showed that the cell growth arrest induced by loss of PRMT5 expression was partially attributable to downregulation of fibroblast growth factor receptor signaling. These results suggest that PRMT5 and its methyltransferase activity is essential for proliferation of lung cancer cells and may serve as a novel target for the treatment of lung cancer.

Keywords

PRMT5; protein arginine methyltransferase; lung cancer; proliferation; FGFR

INTRODUCTION

Protein methylation at arginine residues is catalyzed by protein arginine methyltransferase (PRMT) enzymes [1,2]. PRMT enzymes are evolutionarily conserved in eukaryotes and are classified as type I or type II. One type II enzyme, PRMT5, catalyzes the symmetrical dimethylation of arginine residues within target proteins [2]. PRMT5 is implicated in diverse cellular and biological processes, including transcriptional regulation [3,4,5], RNA metabolism [2,6], ribosome biogenesis [7], Golgi apparatus structure maintenance [8], and cell cycle regulation [3]. In mammalian cells, PRMT5 localizes to both the cytoplasm and the nucleus, and it methylates multiple histones and nonhistone proteins [2]. In the nucleus,

Correspondence: Zhengxin Wang, PhD, Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA. Tel: 713-79-1035; Fax: 713-792-8747; zhenwang@mdanderson.org.

AUTHOR CONTRIBUTIONS

FZ performed orthotopic injection of cells. ZG and SG performed the other experiments. ZQW, WM, and RED performed DNA microarray analysis. ZW wrote the manuscript.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

PRMT5 has been found in the SWI/SNF and NURD chromatin-remodeling complexes [9,10], where it can methylate histones as well as transcription factors or regulators [3,4,5].

In the cytoplasm, PRMT5 forms a 20S protein arginine methyltransferase complex, termed the “methylosome”, consisting of spliceosomal snRNP Sm proteins, PRMT5, pICln, and WD repeat protein (MEP50/WD45) [11,12,13]. In this complex, PRMT5 has been found to methylate Sm proteins [11,14], and such methylation increased the binding affinity of these Sm proteins for the survival motor neuron (SMN), the spinal muscular atrophy disease gene product [15,16]. Subsequently, PRMT5 and SMN complexes cooperate to load the Sm proteins onto U snRNAs, forming U snRNPs [17]. Although in vitro biochemical evidence has indicated that symmetric arginine dimethylation is essential for pre-mRNA splicing [18], the extent to which PRMT5 affects splicing *in vivo* remains elusive.

PRMT5-directed methylation of p53 has been shown to occur in cells that have DNA damage, and this methylation coincided with activation of the p53 response [20]. PRMT5 also methylated epidermal growth factor receptors to promote cell survival and growth [19]. PRMT5 activity was found to be enhanced by cyclin D/Cdk4 kinase, triggering neoplastic growth [21]. Furthermore, PRMT5 controls growth regulation by E2F1 via direct methylation of it [22]. Given these roles, PRMT5 is generally thought to promote tumor growth. Indeed, PRMT5 has been found to be overexpressed in leukemia and lymphoma cells [23,24] and in a subgroup of colorectal cancer cells, in which high levels of PRMT5 and low levels of E2F1 were associated with poor prognosis [22].

Accumulating evidence indicates that fibroblast growth factors (FGFs) and FGF receptors (FGFRs) act in an oncogenic fashion to promote cancer growth and progression. The FGF family consists of 18 ligands that bind to 4 homologous, high-affinity receptors (FGFR1-FGFR4) [25,26]. Ligand (FGF) binding promotes dimerization of FGFRs, enables them to transphosphorylate each other, and triggers downstream signaling events. FGFR signaling plays an essential role in regulating cell proliferation, survival, migration, and differentiation during development and adult life, and deregulation of FGFR signaling has been associated with breast, bladder, prostate, and lung cancers [27]. Therapeutic strategies targeting FGFs and FGFRs in human cancer are therefore currently being explored (http://ClinicalTrials.gov).

In lung cancer, FGFRs have frequently been found to be overactivated [28,29,30,31,32], suggesting that an FGFR-dependent autocrine signaling pathway may operate in lung cancers [32]. Indeed, activated FGFR signaling plays an important role in promoting proliferation of lung cancer cells [29,31,32]. Somatic mutation and amplification of the *FGFR1* gene have been detected in human lung cancer, albeit at a very low frequency [33,34].

Although PRMT5, like FGFR, has been shown to promote tumor growth, and it is overexpressed in some types of cancer cells, its role in the proliferation of lung cancer cells has not been explored. In the present study, we found that PRMT5 was highly expressed in lung tumor samples and lung cancer cell lines but absent in benign lung tissues. Silencing PRMT5 expression in lung adenocarcinoma A549 cells abolished cell growth in tissue cultures and tumor xenografts in nude mice. In addition, PRMT5 regulated the growth of lung cancer cells partially through FGFR signaling. These findings indicate that PRMT5 plays an essential role in the growth of lung cancer.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

EXPERIMENTAL

Lung tumor samples and immunohistochemical analysis of PRMT5 expression

Lung tumor samples and benign lung tissue samples (including alveolar ducts, epithelial cells, and stromal cells surrounding the tumor) were obtained from patients with lung cancer (adenocarcinoma, squamous cell carcinoma, or small-cell lung cancer) who underwent surgery at Tangdu Hospital (Xi'an, China), and the study protocol was approved by its institutional review board. Samples were fixed with 10% formalin for 24 hours and then embedded in paraffin. Paraffin-embedded lung tissue sections (4 μm) were stained with hematoxylin and eosin and used for histologic analysis. The lung tissue sections were blocked with 1% fish gel and incubated with a rabbit polyclonal anti-PRMT5 antibody (1:500; Enzo Life Sciences) overnight at 4°C. A streptavidin-biotin peroxidase detection system for use with prostate tissues (DAKO A/S, Grostrup, Denmark) was used according to the manufacturer's instructions to detect expression levels of PRMT5; 3,3′-diaminobenzidine was used as the substrate.

PRMT5 silencing in lung cancer cells

A549 lung adenocarcinoma cells were cultured in minimum essential medium (Cellgro) with 10% (v/v) fetal bovine serum (HyClone). Short hairpin RNA (shRNA) targeted against the sequence in the coding region of the human *PRMT5* gene (target sequence: 5′-GGATAAAGCTGTATGCTGT-3′) and a nontargeting (NT) control shRNA, whose sequence did not match that of any known human gene (sequence: 5′-TTCTCCGAACGTGTCACGT-3′), were designed with a hairpin and sticky ends (*Cla*I and *Mlu*I). The oligonucleotides were annealed into pLVTHM, a lentiviral gene transfer vector, using the ClaI and MluI restriction enzyme sites. The DNA was sequenced to determine the proper insertion points and insert lengths. The lentivirus was then produced by transfecting 293T human embryonic kidney cells (Invitrogen) with the sequence-verified pLVTHM vector, the packaging plasmid (MD2G), and the envelope plasmid (PAX2) required for viral production. Three days after transfection, the viral supernatant was collected and filtered to remove cellular debris. A549 cells (1×10^5) were plated in 6-well plates and 24 hours later were transduced with the lentivirus. After 16 hours, the virus-containing medium was removed and replaced with a normal growth medium. Three days after infection, cells were split at 1:6 and allowed to grow for 3 days. Whole-cell lysates (20 μg of protein) from the infected cells were subjected to Western blot analysis to determine PRMT5 expression.

Nontargetable PRMT5 expression in lung cancer cells

To create a nontargetable PRMT5, the target sequence of the PRMT5 shRNA (GGATAAAGCTGTATGCTGT) was mutated to GGATAAAattaTATGCTGT. Mutant shRNA-resistant PRMT5 cDNA was subcloned into the lentiviral expression vector dsRed-OG2, and the recombinant lentivirus was produced with 293T cells as described above. To rescue PRMT5 expression, A549 cells (1×10^5) that had transduced with PRMT5 shRNA were plated in 6-well plates and transduced with the virus containing either the nontargetable PRMT5 expression vector or an empty vector. After 48 hours, the cells were replated and PRMT5 expression was determined by Western blot. We also generated nontargetable shRNA-resistant R386A mutant PRMT5 (PRMT5mt) and introduced it into the PRMT5 shRNA-expressing A549 cells. R368 is essential for the methyltransferase activity of PRMT5 [35], and mutation of R368A on PRMT5 has been found to abolish the methyltransferase activity of PRMT5 [36].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Cell culture and cell growth assay

For a cell growth assay, A549 cells infected with lentiviruses containing NT shRNA or PRMT5 shRNA were plated on 24-well plates (5,000 cells per well) and counted every day. For a BrdU incorporation assay, cells (50–70% confluent) were placed on coverslips and cultured with 10 μM BrdU (Sigma-Aldrich) for 4 hours. The BrdU-labeled cells were detected using a monoclonal anti-BrdU antibody stain (BD Biosciences).

Gene expression profiling

Four days after A549 cells were infected with the lentivirus containing NT shRNA or PRMT5 shRNA, total RNA was extracted from the A549 cells using the RNAqueous kit (Ambion). After RNA quality was confirmed using a Bioanalyzer 2100 instrument (Agilent), 300 ng of total RNA was amplified and biotin-labeled using an Eberwine procedure in an Illumina TotalPrep RNA amplification kit (Ambion), and the RNA was then hybridized to Illumina HT12 version 4 human whole-genome microarrays. Processing of bead-level data was by methods previously described [37]. Significance testing for differentially-expressed probes was by the Wilcoxon rank-sum test applied to individual processed bead values, with false-discovery rate significance values (q) determined by the method of Benjamini and Hochberg [38].

Immunohistochemistry

Cultured A549 cells infected with lentiviruses containing NT shRNA or PRMT5 shRNA were allowed to grow on chamber slides and fixed with cold methanol (−20°C) for 10 minutes. Nonspecific proteins were blocked with 4% fish gelatin in PBS for 20 minutes. The cells were incubated with anti-FGFR3 (1:500; Santa Cruz Biotechnology) antibody at 4°C overnight and then incubated with goat anti-rat Alexa 595 (1:500; Invitrogen) at room temperature for 1 hour. The cells were then washed with PBS, counterstained with SYTOX Green (Molecular Probes) for 10 minutes at room temperature, and mounted in Linaris Histogel (Linaris). A fluorescence confocal microscope was used to analyze the cells directly to detect staining for FGFR3.

Orthotopic lung tumors

A549 cells infected with lentiviruses containing NT shRNA, PRMT5 shRNA, or PRMT5 shRNA plus FGFR3 were maintained in minimum essential medium containing 10% fetal bovine serum. Cells in the exponential phase of growth were harvested via treatment with a 0.25% trypsin–2.5% ethylenediaminetetraacetic acid solution for 1 minute, and the resulting cell suspension was gently agitated to produce a single-cell suspension. Cells exhibiting more than 95% viability, which was ascertained using trypan blue staining, were used for injections. Cells were washed with Hank's balanced salt solution and re-suspended in Hank's balanced salt solution diluted with an equal volume of growth factor–reduced Matrigel (Becton Dickinson). Cell suspensions were kept on ice until injection.

Twenty-five 6-week-old nude mice were purchased from the National Cancer Institute and maintained in a barred animal facility. An intraperitoneal injection of sodium pentobarbital (50 mg/kg) was used to anesthetize the mice prior to lung tumor cell injection. Cells (1×10^6/50 μl) were then injected into the left lateral thorax at the lateral dorsal axillary line using a 30-gauge needle. After the tumor cell injection, the mice were turned to the left lateral decubitus position and observed for 45–60 minutes, until they recovered fully. Mice were killed 21 days after tumor cell injection. The lungs of the mice were then removed, evaluated for tumors, and fixed with formaldehyde. Mice were handled in accordance with the guidelines published in the National Institutes of Health Guide for the Care and Use of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Laboratory Animals. The University of Texas MD Anderson Cancer Center's Institutional Animal Care and Use Committee approved all the experimental procedures used for mice.

Statistical analysis

For BrdU incorporation assay, total 500 cells in three non-overlapped field were counted. Data are presented as the means of 3 or more independent experiments ± the standard error of the mean (SEM). A 2-tailed unpaired Student *t* test was used to determine whether differences between control and experiment samples were statistically significant ($P < 0.05$).

RESULTS

PRMT5 expression in lung cancer cells

Western blot analysis revealed that PRMT5 was expressed in immortalized human lung bronchial epithelial cells (Figure 1A, lane 1) and highly expressed in lung adenocarcinoma (A549, PC14, H441, and H322), squamous cell carcinoma (SW900 and H226), and small-cell lung carcinoma (H69) cell lines (Figure 1A, lanes 2–8).

We examined PRMT5 expression in lung tumor samples and benign lung tissue samples from 35 patients (15 with adenocarcinoma, 15 with squamous cell carcinoma, and 5 with small-cell lung carcinoma). Immunostaining experiments revealed that PRMT5 expression was absent from alveolar ducts and benign epithelial cells (Figure 1B). However, strong PRMT5 immunostaining was present in all tumor samples, although it was absent from the stromal cells surrounding the tumors (Figure 1B). These results indicate that expression of PRMT5 may have contributed to lung tumorigenesis.

Silencing PRMT5 expression suppressed lung cancer cell growth

To determine whether PRMT5 plays a causal role in lung tumor growth, we silenced PRMT5 expression in lung adenocarcinoma A549 cells. Specifically, we infected A549 lung cancer cells with a lentiviral vector containing a DNA segment specifying the PRMT5-targeted shRNA sequences or the NT control shRNA sequences. Western blot analysis revealed that PRMT5 shRNA abolished PRMT5 protein expression in A549 cells 4 days after lentiviral infection (Figure 2A, lane 2 versus lane 1). PRMT5 protein levels were partially restored by expression of shRNA-resistant PRMT5 cDNA in PRMT5 shRNA-expressing A549 cells (Figure 2A, lane 3).

Silencing PRMT5 expression with PRMT5 shRNA dramatically inhibited A549 cell growth (Figure 2B, red line). Expression of shRNA-resistant PRMT5 largely restored the growth of PRMT5 shRNA-expressing A549 cells (Figure 2B, yellow line). Amino acid residue R368 is essential for the methyltransferase activity of PRMT5 [35], and mutation of R368A on PRMT5 (PRMT5mt) has been found to abolish the methyltransferase activity of PRMT5 [36]. Expression of shRNA-resistant PRMT5mt failed to restore the growth of PRMT5 shRNA-expressing A549 cells (Figure 2B, green line). These findings suggest that both PRMT5 expression and its methyltransferase activity were required for lung adenocarcinoma A549 cell growth in the tissue culture.

PRMT5 was required for proliferation of lung cancer cells

We used a BrdU incorporation assay to measure the proliferation of A549 cells. The percentage (mean ± SEM) of BrdU-positive NT shRNA-expressing A549 cells (97.0 ± 4.8%; Figure 2C, left panel, in brown, an BrdU-negative cell indicated by the white arrow) was much higher than the percentage of BrdU-positive PRMT5 shRNA-expressing A549 cells (5.1 ± 0.5%; Figure 2C, right panel, BrdU-negative cells indicated by black arrows), indicating that silencing PRMT5 expression suppressed the proliferation of A549 cells.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Flow cytometry analysis of the sub G population revealed that a very small percentage of NT shRNA-expressing A549 cells (0.92%) underwent apoptosis, and silencing PRMT5 only slightly increased the percentage (3.09%) of apoptotic A549 cells (Fig. 3A), indicating that the loss of PRMT5 expression did not significantly induce apoptosis in A549 cells. We next sought to determine whether loss of PRMT5 expression affected the cell cycle progression of A549 cells. Flow cytometry analysis of propidium iodide–stained cells revealed that the proportion of PRMT5 shRNA-expressing lung cancer cells in the G1 phase (63.091%) (Fig. 3B, bottom) was significantly higher than the proportion of NT shRNA-expressing cells in the G1 phase (51.103%) (Fig. 3B, top). In addition, the proportion of PRMT5 cells in the S phase (22.090%) was lower than the proportion of NT shRNA-expressing cells in the S phase (33.132%). Thus, the slow growth of PRMT5 shRNA-expressing cells may be attributed to the arrest of the cell cycle at the G1 phase.

Silencing PRMT5 expression decreased expression of FGFRs

DNA microarray expression profiling analysis revealed that expression of the *FGFR1*, *FGFR3*, and *FGFR4* genes was downregulated and expression of the *FGF17* and *FGFBP1* genes was upregulated in PRMT5 shRNA-expressing A549 cells compared with NT shRNA-expressing A549 cells (Figure 4A). Western blot and immunostaining with the anti-FGFR3 antibody indicated that FGFR3 protein levels were significantly lower in PRMT5 shRNA-expressing A549 cells than in NT shRNA-expressing A549 cells (Figure 4B, lane 2 versus lane 1; Figure 4C, bottom panels versus top panels).

To determine whether loss of FGFR3 expression is responsible for the cell growth inhibition induced by PRMT5 silencing, we overexpressed FGFR3 via lentivirus in A549 cells (Figure 5A, lanes 3 and 4). In the control (empty vector) A549 cells (lanes 1 and 2), PRMT5 silencing abolished FGFR3 expression. In contrast, in FGFR3-expressing A549 cells (lanes 3 and 4), PRMT5 silencing did not alter FGFR3 protein levels. Ectopic expression of FGFR3 partially restored cell growth inhibited by PRMT5 silencing (Figure 5B). These results indicate that one mechanism by which PRMT5 controls cell growth is through the regulation of FGFR3 expression.

PRMT5 expression was essential for A549 cell growth in an orthotopic mouse model

To determine the effects of PRMT5 silencing on lung cancer, we observed the growth in mice of orthotopic tumors formed from A549 cells expressing NT shRNA, PRMT5 shRNA, or PRMT5 shRNA plus FGFR3. Large, macroscopically visible tumors were found in the lungs of the 9 mice injected with NT shRNA-expressing A549 cells (Figure 6A, top panel). However, no tumors were detected in the lungs of the 8 mice injected with PRMT5 shRNA-expressing A549 cells (Figure 6A, middle panel). We found small tumors in the lungs of 5 of the 8 mice injected with PRMT5 shRNA plus FGFR3-expressing A549 cells (Figure 6A, bottom panel, indicated by black arrows). The average size (mean ± SEM) of the lung tumors in the mice injected with NT shRNA-expressing A549 cells (13.3 ± 4.3 mm^2) was much higher than that of the lung tumors in mice injected with PRMT5 shRNA plus FGFR3-expressing A549 cells (2.6 ± 1.4 mm^2; Figure 6B). These results indicate that PRMT5 is essential for growth of lung tumor xenografts, and that the growth occurs partially through the FGFR3 signaling.

DISCUSSION

Our findings indicate that PRMT5 plays an essential role in the growth of lung cancer. This was demonstrated in our analysis of lung cancer patient tissue samples, in which we detected high expression levels of PRMT5 in lung cancer samples and no PRMT5 expression in benign lung tissue samples. The role of PRMT5 in lung cancer cell growth was also evident

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

in our *in vitro* and *in vivo* studies: PRMT5 silencing dramatically inhibited the growth of lung adenocarcinoma A549 cells in tissue cultures and abolished the growth of lung A549 xenografts in nude mice. These findings also suggest that PRMT5 expression and its downstream effectors could be novel targets for the treatment of lung cancer.

We also found that silencing PRMT5 expression decreased cell proliferation and led to cell cycle arrest at the G1 phase, which is supported by previous findings. E2F transcriptional factors play a major role in the regulation of cell-cycle progression from the G1 to S phase [39,40,41]. One recent study demonstrated that PRMT5 directly methylates E2F1 and that the arginine methylation is responsible for regulating E2F1's biochemical and functional properties [42]. These findings raised the possibility that arginine methylation may contribute to cell cycle progression influenced by E2F1.

Previous published studies have also demonstrated a critical role for FGFR signaling in the proliferation of lung cancer cells [29,31,32]. Somatic mutations and amplification of the FGFR1 gene have been detected in human lung cancer, albeit at a very low frequency [33,34]. Thus, activation of FGFR signaling in lung cancer might occur through upregulation of transcription of genes of FGFs or FGFRs. In the current study, DNA microarray analysis revealed a decrease in expression of FGFRs in the PRMT5 shRNA-expressing A549 cells. Expression of some negative regulators of FGFR signaling (SPRY2, SPRY4, and FLRT3) [43,44] was also increased in the PRMT5 shRNA-expressing A549 cells (Figure 4A). Furthermore, expression of neuronal precursor cell-expressed developmentally downregulated 4 (Nedd4) more than doubled in the PRMT5 shRNA-expressing A549 cells (Figure 4A). Nedd4 is an E3 ubiquitin ligase that suppresses FGFR signaling via the ubiquitin-mediated degradation pathway [45]. Decreased SPRY and FLRT3 expression and increased Nedd4 expression might lead to significantly decreased FGFR signaling in the PRMT5 shRNA-expressing A549 cells. This suggests that PRMT5 regulates expression of FGFRs directly and indirectly, providing a novel mechanism by which FGFRs are activated in lung cancer.

The role of PRMT5 in FGFR signaling regulation is further supported by our finding that ectopic expression of FGFR3 partially restored the growth defect of A549 cells and tumor xenografts expressing PRMT5 shRNA. However, ectopic expression of FGFR3, even at high levels, failed to fully reverse the growth inhibition induced by PRMT5 silencing. Thus, additional signaling might be needed to mediate the functions of PRMT5 in A549 cells. This is worthy of further study.

Indeed, the way in which PRMT5 expression is activated in lung cancer cells remains unknown. Future research will focus on the regulatory networks controlling PRMT5 expression in lung cancer cells, which may provide some clues about lung tumorigenesis. It should also be noted that the current study used a lung adenocarcinoma cell line (A549) for most of the experiments. Thus, our results suggest that PRMT5 contributes to lung adenocarcinoma development, but further studies are needed to determine whether PRMT5 also contributes to the development of other subtypes of lung cancers.

Acknowledgments

We thank Erica A Goodoff in MD Anderson's Department of Scientific Publications for editing the manuscript.

FUNDING

This work was supported in part by the National Institutes of Health through MD Anderson's Cancer Center Support Grant (CA16672), the National Natural Science Foundation of China (81171922), and Key Project Research Fund of Shaanxi Provincial Science and Technology Program, China (2008K27G01). No additional

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

external funding for this study was received. The funding agency had no role in study design, data collection or analysis, decision to publish, or manuscript preparation.

Abbreviation

FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FLRT3	leucine-rich repeat transmembrane protein 3
NEDD4	neural precursor cell expressed developmentally down-regulated protein 4
NT shRNA	non-target small hairpin RNA
PRMT5	protein arginine methyltransferase 5
shRNA	small hairpin RNA
SmD3	Sm protein D3
SPRY	sprouty homolog

References

1. Wolf SS. The protein arginine methyltransferase family: an update about function, new perspectives and the physiological role in humans. Cellular and molecular life sciences: CMLS. 2009; 66:2109–21. [PubMed: 19300908]
2. Bedford MT, Clarke SG. Protein arginine methylation in mammals: who, what, and why. Mol Cell. 2009; 33:1–13. [PubMed: 19150423]
3. Jansson M, Durant ST, Cho EC, Sheahan S, Edelmann M, et al. Arginine methylation regulates the p53 response. Nat Cell Biol. 2008; 10:1431–9. [PubMed: 19011621]
4. Bedford MT, Richard S. Arginine methylation an emerging regulator of protein function. Mol Cell. 2005; 18:263–72. [PubMed: 15866169]
5. Amente S, Napolitano G, Licciardo P, Monti M, Pucci P, et al. Identification of proteins interacting with the RNAPII FCP1 phosphatase: FCP1 forms a complex with arginine methyltransferase PRMT5 and it is a substrate for PRMT5-mediated methylation. FEBS Lett. 2005; 579:683–9. [PubMed: 15670829]
6. Liu C, Lu F, Cui X, Cao X. Histone methylation in higher plants. Annu Rev Plant Biol. 2010; 61:395–420. [PubMed: 20192747]
7. Ren J, Wang Y, Liang Y, Zhang Y, Bao S, et al. Methylation of ribosomal protein S10 by protein-arginine methyltransferase 5 regulates ribosome biogenesis. J Biol Chem. 2010; 285:12695–705. [PubMed: 20159986]
8. Zhou Z, Sun X, Zou Z, Sun L, Zhang T, et al. PRMT5 regulates Golgi apparatus structure through methylation of the golgin GM130. Cell Res. 2010; 20:1023–33. [PubMed: 20421892]
9. Le Guezennec X, Vermeulen M, Brinkman AB, Hoeijmakers WA, Cohen A, et al. MBD2/NuRD and MBD3/NuRD, two distinct complexes with different biochemical and functional properties. Mol Cell Biol. 2006; 26:843–51. [PubMed: 16428440]
10. Pal S, Vishwanath SN, Erdjument-Bromage H, Tempst P, Sif S. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. Mol Cell Biol. 2004; 24:9630–45. [PubMed: 15485929]
11. Friesen WJ, Paushkin S, Wyce A, Massenet S, Pesiridis GS, et al. The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. Mol Cell Biol. 2001; 21:8289–300. [PubMed: 11713266]
12. Meister G, Eggert C, Buhler D, Brahms H, Kambach C, et al. Methylation of Sm proteins by a complex containing PRMT5 and the putative U snRNP assembly factor pICln. Curr Biol. 2001; 11:1990–4. [PubMed: 11747828]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

13. Friesen WJ, Wyce A, Paushkin S, Abel L, Rappsilber J, et al. A novel WD repeat protein component of the methylosome binds Sm proteins. J Biol Chem. 2002; 277:8243–7. [PubMed: 11756452]
14. Brahms H, Meheus L, de Brabandere V, Fischer U, Luhrmann R. Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B′ and the Sm-like protein LSm4, and their interaction with the SMN protein. RNA. 2001; 7:1531–42. [PubMed: 11720283]
15. Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss GSMN. The product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. Mol Cell. 2001; 7:1111–7. [PubMed: 11389857]
16. Meister G, Fischer U. Assisted RNP assembly: SMN and PRMT5 complexes cooperate in the formation of spliceosomal UsnRNPs. EMBO J. 2002; 21:5853–63. [PubMed: 12411503]
17. Neuenkirchen N, Chari A, Fischer U. Deciphering the assembly pathway of Sm-class U snRNPs. FEBS Lett. 2008; 582:1997–2003. [PubMed: 18348870]
18. Boisvert FM, Cote J, Boulanger MC, Cleroux P, Bachand F, et al. Symmetrical dimethylarginine methylation is required for the localization of SMN in Cajal bodies and pre-mRNA splicing. J Cell Biol. 2002; 159:957–69. [PubMed: 12486110]
19. Hsu JM, Chen CT, Chou CK, Kuo HP, Li LY, et al. Crosstalk between Arg 1175 methylation and Tyr 1173 phosphorylation negatively modulates EGFR-mediated ERK activation. Nature cell biology. 2011; 13:174–81.
20. Jansson M, Durant ST, Cho EC, Sheahan S, Edelmann M, et al. Arginine methylation regulates the p53 response. Nature cell biology. 2008; 10:1431–9.
21. Aggarwal P, Vaites LP, Kim JK, Mellert H, Gurung B, et al. Nuclear cyclin D1/CDK4 kinase regulates CUL4 expression and triggers neoplastic growth via activation of the PRMT5 methyltransferase. Cancer cell. 2010; 18:329–40. [PubMed: 20951943]
22. Cho EC, Zheng S, Munro S, Liu G, Carr SM, et al. Arginine methylation controls growth regulation by E2F-1. EMBO J. 2012; 31:1785–97. [PubMed: 22327218]
23. Pal S, Baiocchi RA, Byrd JC, Grever MR, Jacob ST, et al. Low levels of miR-92b/96 induce PRMT5 translation and H3R8/H4R3 methylation in mantle cell lymphoma. EMBO J. 2007; 26:3558–69. [PubMed: 17627275]
24. Wang L, Pal S, Sif S. Protein arginine methyltransferase 5 suppresses the transcription of the RB family of tumor suppressors in leukemia and lymphoma cells. Molecular and cellular biology. 2008; 28:6262–77. [PubMed: 18694959]
25. Wesche J, Haglund K, Haugsten EM. Fibroblast growth factors and their receptors in cancer. The Biochemical Journal. 2011; 437:199–213. [PubMed: 21711248]
26. Mendelson CR. Role of transcription factors in fetal lung development and surfactant protein gene expression. Annu Rev Physiol. 2000; 62:875–915. [PubMed: 10845115]
27. Ribatti D, Vacca A, Rusnati M, Presta M. The discovery of basic fibroblast growth factor/ fibroblast growth factor-2 and its role in haematological malignancies. Cytokine & growth factor reviews. 2007; 18:327–34. [PubMed: 17537668]
28. Behrens C, Lin HY, Lee JJ, Raso MG, Hong WK, et al. Immunohistochemical expression of basic fibroblast growth factor and fibroblast growth factor receptors 1 and 2 in the pathogenesis of lung cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2008; 14:6014–22. [PubMed: 18829480]
29. Berger W, Setinek U, Mohr T, Kindas-Mugge I, Vetterlein M, et al. Evidence for a role of FGF-2 and FGF receptors in the proliferation of non-small cell lung cancer cells. International journal of cancer. Journal international du cancer. 1999; 83:415–23. [PubMed: 10495436]
30. Chandler LA, Sosnowski BA, Greenlees L, Aukerman SL, Baird A, et al. Prevalent expression of fibroblast growth factor (FGF) receptors and FGF2 in human tumor cell lines. International journal of cancer. Journal international du cancer. 1999; 81:451–8. [PubMed: 10209961]
31. Kuhn H, Kopff C, Konrad J, Riedel A, Gessner C, et al. Influence of basic fibroblast growth factor on the proliferation of non-small cell lung cancer cell lines. Lung cancer. 2004; 44:167–74. [PubMed: 15084381]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

32. Marek L, Ware KE, Fritzsche A, Hercule P, Helton WR, et al. Fibroblast growth factor (FGF) and FGF receptor-mediated autocrine signaling in non-small-cell lung cancer cells. Molecular pharmacology. 2009; 75:196–207. [PubMed: 18849352]
33. Zhao X, Weir BA, LaFramboise T, Lin M, Beroukhim R, et al. Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. Cancer Research. 2005; 65:5561–70. [PubMed: 15994928]
34. Davies H, Hunter C, Smith R, Stephens P, Greenman C, et al. Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Research. 2005; 65:7591–5. [PubMed: 16140923]
35. Pollack BP, Kotenko SV, He W, Izotova LS, Barnoski BL, et al. The human homologue of the yeast proteins Skb1 and Hsl7p interacts with Jak kinases and contains protein methyltransferase activity. J Biol Chem. 1999; 274:31531–42. [PubMed: 10531356]
36. Hosohata K, Li P, Hosohata Y, Qin J, Roeder RG, et al. Purification and identification of a novel complex which is involved in androgen receptor-dependent transcription. Mol Cell Biol. 2003; 23:7019–29. [PubMed: 12972618]
37. Ma W, Wang M, Wang ZQ, Sun L, Graber D, et al. Effect of Long-Term Storage in Trizol on Microarray-Based Gene Expression Profiling. Cancer Epidemiol Biomarkers Prev. 2010; 19:2445–52. [PubMed: 20805315]
38. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B. 1995; 57:289–300.
39. Classon M, Harlow E. The retinoblastoma tumour suppressor in development and cancer. Nat Rev Cancer. 2002; 2:910–7. [PubMed: 12459729]
40. Cobrinik D. Pocket proteins and cell cycle control. Oncogene. 2005; 24:2796–809. [PubMed: 15838516]
41. Gonzalo S, Blasco MA. Role of Rb family in the epigenetic definition of chromatin. Cell Cycle. 2005; 4:752–5. [PubMed: 15908781]
42. Cho EC, Zheng S, Munro S, Liu G, Carr SM, et al. Arginine methylation controls growth regulation by E2F-1. The EMBO journal. 2012; 31:1785–97. [PubMed: 22327218]
43. Martinez N, Garcia-Dominguez CA, Domingo B, Oliva JL, Zarich N, et al. Sprouty2 binds Grb2 at two different proline-rich regions, and the mechanism of ERK inhibition is independent of this interaction. Cellular signalling. 2007; 19:2277–85. [PubMed: 17689925]
44. Bottcher RT, Pollet N, Delius H, Niehrs C. The transmembrane protein XFLRT3 forms a complex with FGF receptors and promotes FGF signalling. Nature Cell Biology. 2004; 6:38–44.
45. Persaud A, Alberts P, Amsen EM, Xiong X, Wasmuth J, et al. Comparison of substrate specificity of the ubiquitin ligases Nedd4 and Nedd4-2 using proteome arrays. Molecular Systems Biology. 2009; 5:333. [PubMed: 19953087]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

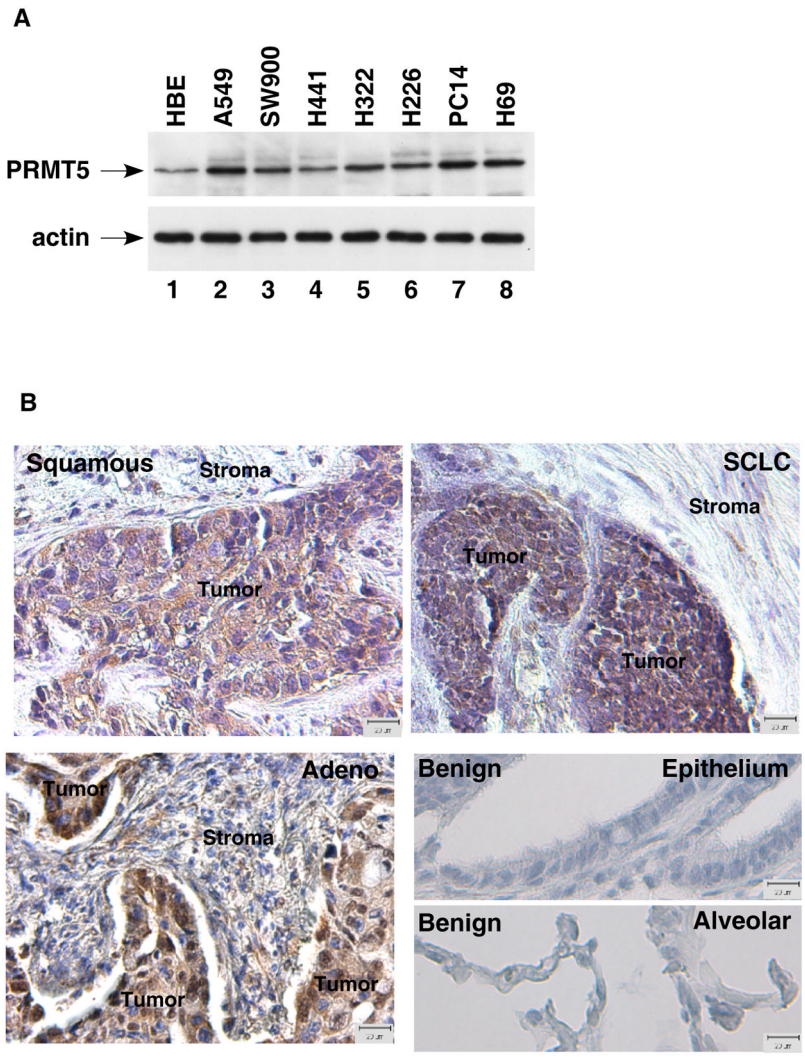

Figure 1. PRMT5 expression is associated with lung cancer tumorigenesis

(**A**) Western blot analysis of PRMT5 expression in whole cell lysates (10 μg proteins per sample) derived from immortalized human lung epithelial cells (HBE; lane 1) and lung cancer cell lines (lanes 2–8). (**B**) Immunostaining for PRMT5 in lung cancer and benign lung tissue samples. Adeno, adenocarcinoma; squamous, squamous cell carcinoma; SCLC, small-cell lung carcinoma. Original amplification is 100 and the scale bar represents 20 μm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

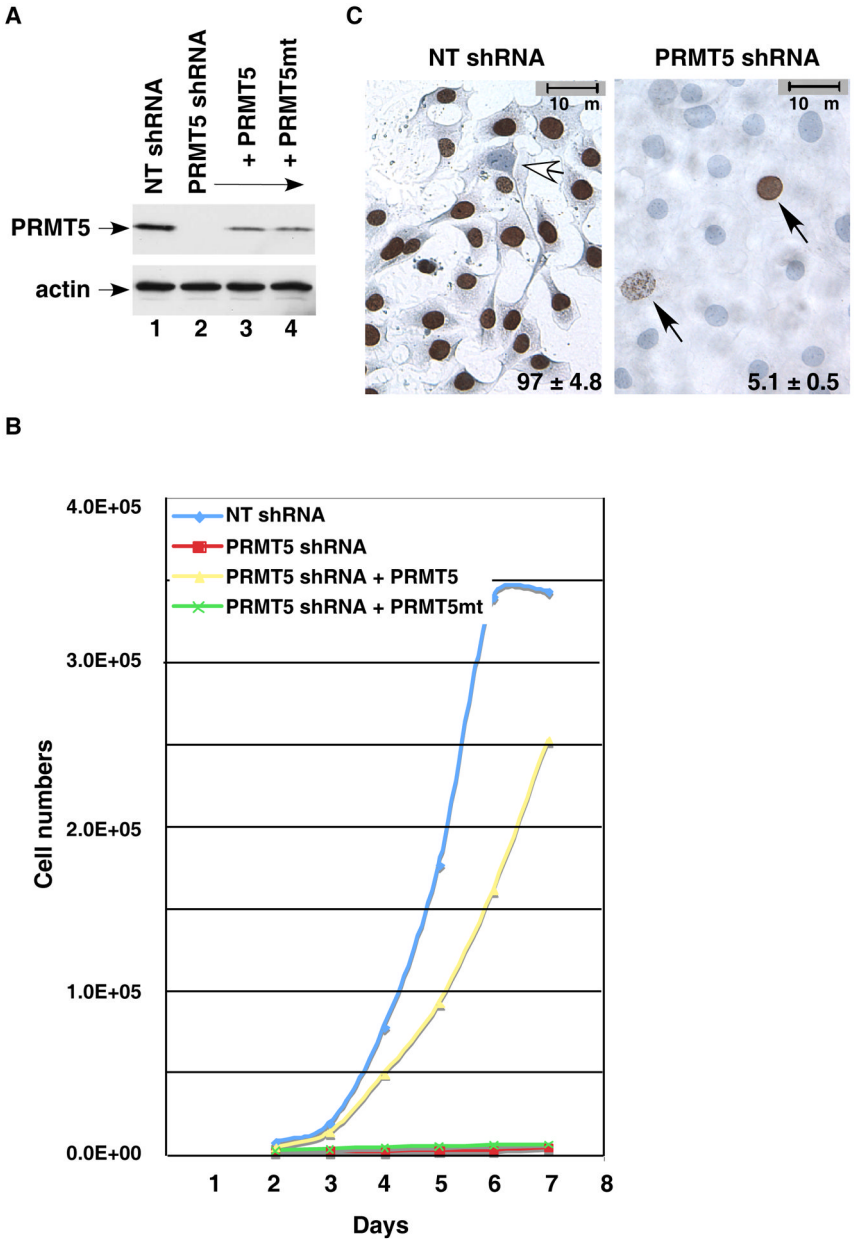

Figure 2. Silencing of PRMT5 expression inhibits lung cancer cell growth

(**A**) Short hairpin RNA (shRNA)-mediated silencing of PRMT5 expression in lung cancer cells shown by Western blot analysis of whole cell lysates from A549 cells infected with a lentivirus expressing nontargeting (NT) shRNA (lane 1), PRMT5 shRNA (lane 2), PRMT5 shRNA plus shRNA-resistant PRMT5 (lane 3), and PRMT5 shRNA plus shRNA-resistant mutant PRMT5 (PRMT5mt; lane 4). (**B**) Growth curves of lung cancer cells expressing NT shRNA, PRMT5 shRNA, PRMT5 shRNA plus shRNA-resistant PRMT5, and PRMT5 shRNA plus shRNA-resistant PRMT5mt. (**C**) A549 cells infected with NT shRNA- or PRMT5 shRNA-expressing lentivirus were allowed to grow in the presence of BrdU and immunostained with an anti-BrdU antibody (brown). The white arrow (left panel) indicated the BrdU-negative staining cell and the black arrows (right panel) indicated the BrdU-positive staining cells. Original amplification is 200 and the scale bar represents 10 μm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

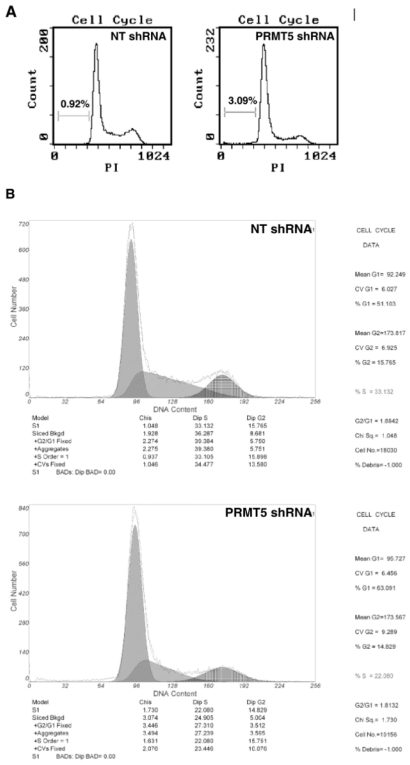

Figure 3. Silencing PRMT5 expression arrested cell cycle at G1 phase

(**A**) A549 cells expressing nontargeting NT shRNA (left) or PRMT5 shRNA (left) were submitted to flow cytometric analysis of sub-G1 content of cells. Percentage of cells that were undergoing apoptosis was indicated. (**B**) A549 cells expressing nontargeting NT shRNA (top) or PRMT5 shRNA (bottom) were submitted to flow cytometric analysis of cell cycle progression. Percentage of cells that were at G1, G2, or S phase was indicated on the left.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

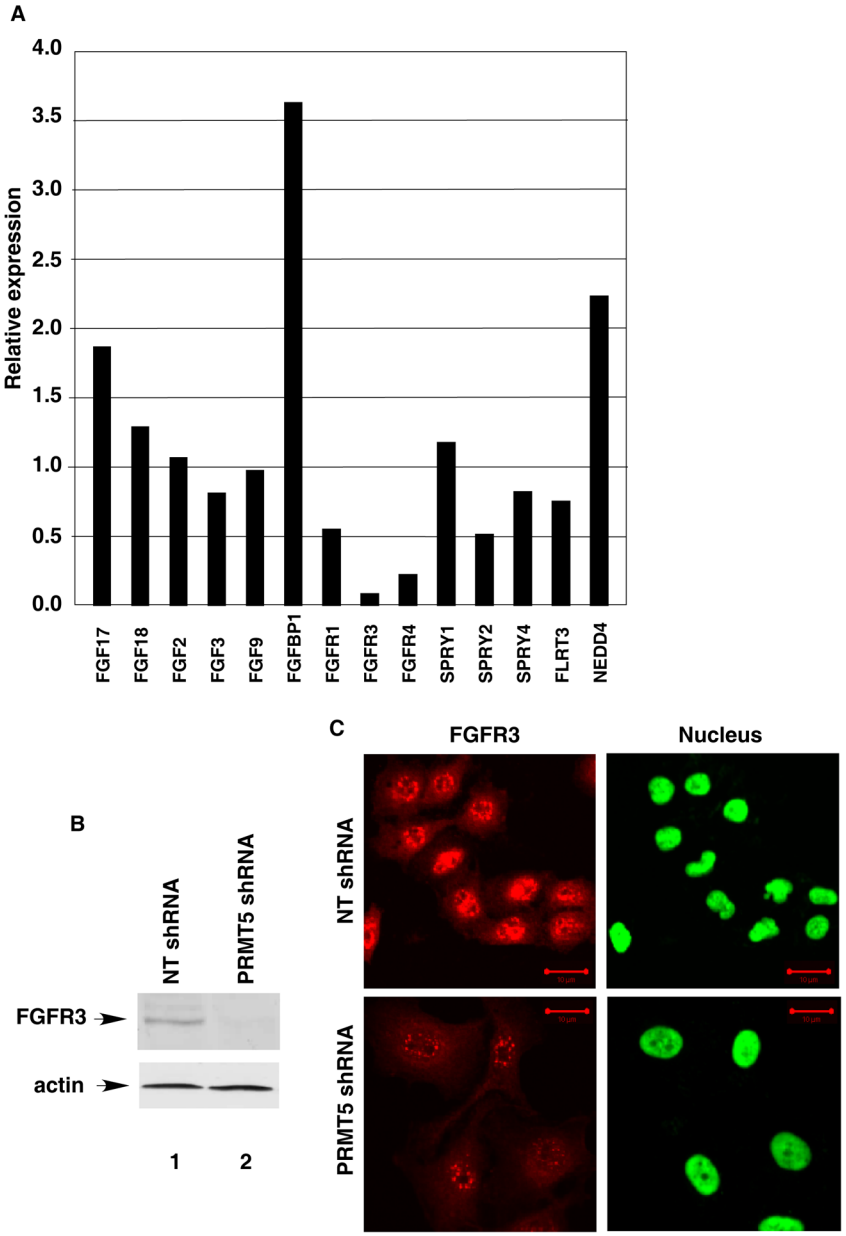

Figure 4. Silencing PRMT5 expression decreased fibroblast growth factor receptor 3 (FGFR3) expression

(**A**) Expression of FGFs, FGFRs, and negative regulators of FGFR signaling (SPRY1, SPRY2, SPRY4, FLRT3, and NEDD4) in A549 cells expressing nontargeting (NT) short hairpin RNA (shRNA) or PRMT5 shRNA. Relative expression = relative signals in PRMT5 shRNA-expressing cells/relative signals in NT shRNA-expressing cells. (**B**) Western blot analysis of whole-cell lysates derived from A549 cells expressing NT shRNA or PRMT5 shRNA with anti-FGFR3 or anti-actin antibodies. (**C**) Immunostaining for FGFR3 in A549 cells expressing NT shRNA (top) or PRMT5 shRNA (bottom). Original amplification is 400 and the scale bar represents 10 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

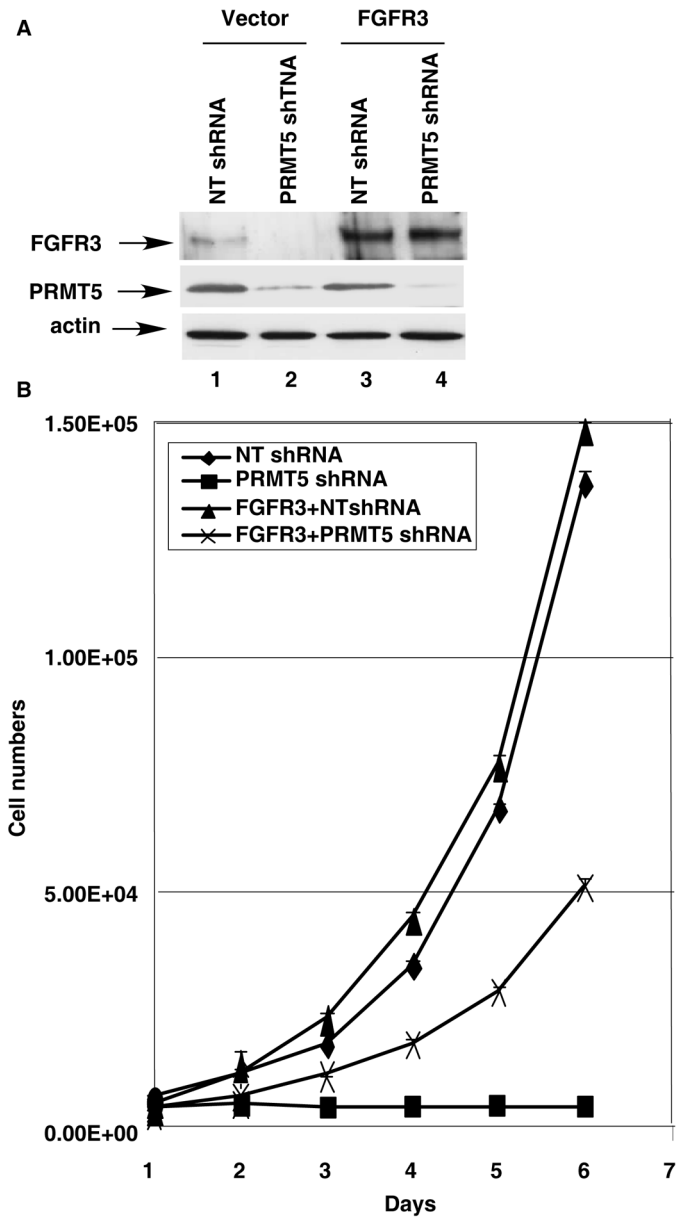

Figure 5. PRMT5 targets fibroblast growth factor receptor 3 (FGFR3) to control lung cancer cell growth

(**A**) Western blot analysis of whole-cell lysates derived from A549 cells expressing nontargeting (NT) short hairpin RNA (shRNA; lane 1), NT shRNA plus FGFR3 (lane 3), PRMT5 shRNA (lane 2), and PRMT5 shRNA plus FGFR3 (lane 4), with anti-FGFR3 (top), anti-PRMT5 (middle), or anti-actin (bottom) antibodies. (**B**) Growth curves of A549 cells expressing NT shRNA, NT shRNA plus FGFR3, PRMT5 shRNA, and PRMT5 shRNA plus FGFR3.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

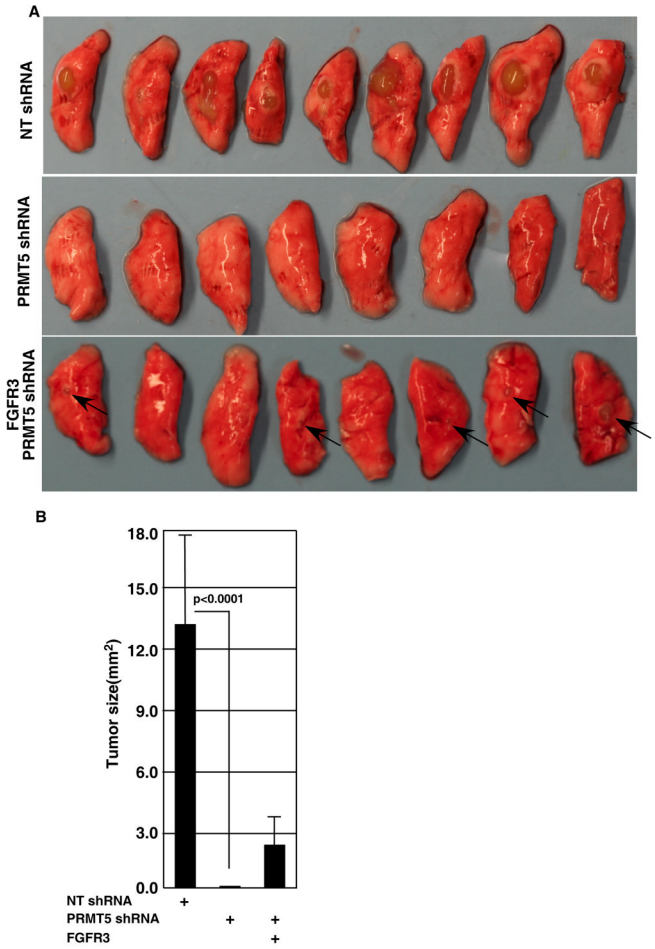

Figure 6. Loss of PRMT5 expression abolished growth of lung tumor xenografts

(**A**) Lungs derived from mice injected with A549 cells stably expressing nontargeting (NT) short hairpin RNA (shRNA; top), PRMT5 shRNA (middle), or PRMT5 shRNA plus fibroblast growth factor receptor 3 (FGFR3; bottom). Small tumors in lungs injected with A549 cells stably expressing PRMT5 shRNA plus fibroblast growth factor receptor 3 (FGFR3; bottom) are indicated by black arrows. (**B**) Mean size of tumors in mouse lungs injected with A549 cells stably expressing NT shRNA, PRMT5 shRNA, or PRMT5 shRNA plus FGFR3. Vertical bars indicate SEM.