INTRODUCTION. MicroRNAs (miRNAs) are small, noncoding RNAs that regulate expression of many genes. Recent studies suggest roles of miRNAs in carcinogenesis. We and others have shown that expression profiles of miRNAs are different in lung cancer vs. normal lung, although the significance of this aberrant expression is poorly understood.

METHODS. qRT-PCR. qRT-PCR analysis for miRNAs was performed in triplicate with the TaqMan MicroRNA assays kit (Applied Biosystems, Foster City, CA) according to the instructions of the manufacturer, and 18S RNA was used for normalization; qRT-PCR analyses for other genes of interest were performed as described previously.

Luciferase Reporter Assay for Targeting DNMT 3'-UTRs. For Luciferase reporter experiments, a DNMT3A 3'-UTR segment of 979 bp and a DNMT3B 3'-UTR segment of 978 bp were amplified by PCR from human genomic DNA and inserted into the pGL3-control vector with simian virus 40 promoter (Promega, Madison, WI) by using the XbaI site immediately downstream from the stop codon of luciferase.

GFP Repression Constructs to Assess Effect of DNMT 3'-UTRs on Protein Expression. For GFP repression, a DNMT3A 3'-UTR segment of 1472 bp and a DNMT3B 3'-UTR segment of 1566 bp (corresponding to the entire length of the 3'-UTRs) were amplified by PCR from human genomic DNA and inserted into the AFP pQBi25F vector (Qbiogene, Irvine, CA) by using the BamHI-Hind III cloning sites located 3' of the GFP encoding sequence of the vector (which has no stop codon at the end of the GFP coding sequence).

RESULTS. Among the reported down-regulated miRNAs in lung cancer, the miRNA (miR)-29 family (29a, 29b and 29c) has intriguing complementarities to the 3'-UTRs of DNA methyltransferase (DNMT)3A and -3B (de novo methyltransferases), two key enzymes involved in DNA methylation, that are frequently up-regulated in lung cancer and associated with poor prognosis. We investigated whether miR-29s could target DNMT3A and -B and whether restoration of miR-29s could normalize aberrant patterns of methylation in non-small-cell lung cancer. We show that expression of miR-29s is inversely correlated to DNMT3A and -3B in lung cancer tissues, and that mir-29s directly target both DNMT3A and -3B. The enforced expression of miR-29s in lung cancer cell lines restores normal patterns of DNA methylation, induces re-expression of methylation-silenced tumor suppressor genes, such as FHIT and
WWOX, and inhibits tumorigenicity in vitro and in vivo. These findings support a role of miR-29s in epigenetic normalization of NSCLC, providing a rationale for the development of miRNA-based strategies for the treatment of lung cancer.

**DISCUSSION.** In summary, this study has shown that expression of miR-29 family members is inversely correlated with DNMT3A and -3B expression in lung cancers and these miRNAs down-modulate expression levels of both enzymes. Furthermore, enforced expression of these miRNAs in lung cancer cells leads to reduced global DNA methylation, restores expression of TSGs and inhibits tumorigenicity both in vitro and in vivo. Results of this study provide a strong rationale for developing epigenetic therapies using synthetic miR-29s, alone or in combination with other treatments, to reactivate tumor suppressors and normalize aberrant patterns of methylation in lung cancer. Because loss of expression of miR-29 family members is observed in other common human malignancies, this approach may be extended to the treatment of other human malignancies.