PRMT5 (protein arginine methyltransferase 5) is an enzyme that catalyses transfer of methyl groups from S-adenosyl methionine to the arginine residues of histones or non-histone proteins and is involved in a variety of cellular processes. Although it is highly expressed in some tumours, its direct role in cancer growth has not been fully investigated. In the present 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 down-regulation of fibroblast growth factor receptor signalling. 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.

Key words: fibroblast growth factor receptor (FGFR), lung cancer, proliferation, protein arginine methyltransferase 5 (PRMT5).

INTRODUCTION

Protein methylation at arginine residues is catalysed by PRMT (protein arginine methyltransferase) enzymes [1,2]. PRMT enzymes are evolutionarily conserved in eukaryotes and are classified as type I or type II. One type II enzyme, PRMT5, catalyses the symmetrical dimethylation of arginine residues within target proteins [2]. PRMT5 is implicated in diverse cellular and biological processes, including transcriptional regulation [3–5], 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 non-histone proteins [2]. In the nucleus, PRMT5 has been found in the SWI/SNF and NuRD (nucleosome remodelling and deacetylase) chromatin-remodelling complexes [9,10], where it can methylate histones as well as transcription factors or regulators [3–5].

In the cytoplasm, PRMT5 forms a 20S protein arginine methyltransferase complex, termed the ‘methylosome’, consisting of spliceosomal snRNPs (small nuclear ribonucleoprotein) Sm proteins, PRMT5, pICln and WD repeat protein [MEP50 (methylsosome protein 50)/WD40] [11–13]. 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 SMN (survival motor neuron), the spinal muscular atrophy disease gene product [15,16]. Subsequently, PRMT5 and SMN complexes co-operate to load the Sm proteins on to U snRNAs (small nuclear RNAs), 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 [3]. 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 (cyclin-dependent kinase 4) 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 tumour growth. Indeed, PRMT5 has been found to be overexpressed in leukaemia 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 FGFs (fibroblast growth factors) and FGFRs (FGF receptors) act in an oncogenic fashion to promote cancer growth and progression. The FGF family consists of 18 ligands that bind to four homologous high-affinity receptors (FGFR1–FGFR4) [25,26]. Ligand (FGF) binding promotes dimerization of FGFRs, enables them to transphosphorylate each other, and triggers downstream signalling events. FGFR signalling plays an essential role in regulating cell proliferation, survival, migration and differentiation during development and adult life, and deregulation of FGFR signalling 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 [28–32], suggesting that an FGFR-dependent autocrine signalling pathway may operate in lung cancers [32]. Indeed, activated FGFR signalling 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 tumour growth, and 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 tumour 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 tumour xenografts in nude mice. In addition, PRMT5 regulated the growth of lung cancer cells partially through FGFR signalling. These findings indicate that PRMT5 plays an essential role in the growth of lung cancer.

EXPERIMENTAL

Lung tumour samples and immunohistochemical analysis of PRMT5 expression

Lung tumour samples and benign lung tissue samples (including alveolar ducts, epithelial cells and stromal cells surrounding the tumour) 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 h and then embedded in paraffin. Paraffin-embedded lung tissue sections (4 μm) were stained with haematoxylin and eosin and used for histological analysis. The lung tissue sections were blocked with 1% fish gel and incubated with a rabbit polyclonal anti-PRMT5 antibody (1:500 dilution; Enzo Life Sciences) overnight at 4°C. A streptavidin–biotin peroxidase detection system for use with prostate tissues (DAKO) 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 and A549 (Cellgro) with 10% fetal bovine serum (HyClone). shRNA (short hairpin RNA) targeted against the sequence in the coding region of the human PRMT5 gene (target sequence: 5′-GGATAAGCTGTATGGCTG-3′) and a NT (non-targeting) control shRNA, whose sequence did not match that of any known human gene (5′-TTCTCAGACGTCACGT-3′), were designed with a hairpin and sticky ends (ClaI and MluI). The oligonucleotides were annealed into plLVTHM, 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 HEK (human embryonic kidney)-293T cells (Invitrogen) with the sequence-verified plLVTHM vector, the packaging plasmid (MD2G) and the envelope plasmid (PAX2) required for viral production. At 3 days after transfection, the viral supernatant was collected and filtered to remove cellular debris. A549 cells (1 × 10⁴) were plated in six-well plates and 24 h later were transduced with the lentivirus. After 16 h, the virus-containing medium was removed and replaced with a normal growth medium. At 3 days after infection, cells were split at a ratio of 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.

Non-targetable PRMT5 expression in lung cancer cells

To create a non-targetable PRMT5, the target sequence of the PRMT5 shRNA (GGATAAAGCTGTATGGCTG) was mutated to GGATATAAAtaTATGGCTG. Mutant shRNA-resistant PRMT5 cDNA was subcloned into the lentiviral expression vector dsRed-O2, and the recombinant lentivirus was produced with HEK-293T cells as described above. To rescue PRMT5 expression, A549 cells (1 × 10⁶) that had been transduced with PRMT5 shRNA were plated in six-well plates and transduced with the virus containing either the non-targetable PRMT5 expression vector or an empty vector. After 48 h, the cells were replated and PRMT5 expression was determined by Western blotting. We also generated non-targetable shRNA-resistant R386A mutant PRMT5 (PRMT5mt) and introduced it into the PRMT5 shRNA-expressing A549 cells. Arg⁶⁰ 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].

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 (5000 cells per well) and counted every day. For a BrdU (bromodeoxyuridine) incorporation assay, cells (50–70% confluent) were placed on to coverslips and cultured with 10 μM BrdU (Sigma–Aldrich) for 4 h. The BrdU-labelled cells were detected using a monoclonal anti-BrdU antibody stain (BD Biosciences).

Gene expression profiling

At 4 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 RNAeasy kit (Ambion). After RNA quality was confirmed using a Bioanalyzer 2100 instrument (Agilent), 300 ng of total RNA was amplified and biotin-labelled 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 described previously [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 min. Non-specific proteins were blocked with 4% fish gelatin in PBS for 20 min. The cells were incubated with anti-FGFR3 antibody (1:500 dilution; Santa Cruz Biotechnology) at 4°C overnight and then incubated with goat anti-(rat Alexa 595) antibody (1:500 dilution; Invitrogen) at room temperature (25°C) for 1 h. The cells were then washed with PBS, counterstained with SYTOX Green (Molecular Probes) for 10 min at room temperature and mounted in Linaris Histogel (Linaris). A fluorescence confocal microscope was used to analyse the cells directly to detect staining for FGFR3.

Orthotopic lung tumours

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.
serum. Cells in the exponential phase of growth were harvested via treatment with a 0.25% trypsin/2.5% ethylenediaminetetraacetic acid solution for 1 min, 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. The cells were washed with Hank’s balanced salt solution and resuspended 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.

Nude mice (25 6-week-old) 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 anaesthetize the mice prior to lung tumour cell injection. Cells (1 × 10⁶/50 μl) were then injected into the left lateral thorax at the lateral dorsal axillary line using a 30-gauge needle. After the tumour cell injection, the mice were turned to the left lateral decubitus position and observed for 45–60 min, until they recovered fully. Mice were killed 21 days after tumour cell injection. The lungs of the mice were then removed, evaluated for tumours 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 Laboratory Animals. The University of Texas MD Anderson Cancer Center’s Institutional Animal Care and Use Committee approved all of the experimental procedures used for mice.

Statistical analysis

For the BrdU incorporation assay, a total of 500 cells in three non-overlapped fields were counted. Data are presented as the means of three or more independent experiments ± S.E.M. A 2-tailed unpaired Student’s 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 tumour 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 of the tumour samples, although it was absent from the stromal cells surrounding the tumours (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 tumour 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 compared with 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 R368A is essential for the methyltransferase activity of PRMT5 [35], and the mutation 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 (means ± S.E.M.) of BrdU-positive NT shRNA-expressing A549 cells (97.0 ± 4.8%); Figure 2C, left-hand panel, in brown; a BrdU-negative cell is 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-hand panel, BrdU-negative cells indicated by
Figure 2  Silencing of PRMT5 expression inhibits lung cancer cell growth
(A) 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 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-hand panel) indicates the BrdU-negative staining cell and the black arrows (right-hand panel) indicate the BrdU-positive staining cells. Original magnification is ×200 and the scale bar represents 10 μm.

black arrows), indicating that silencing PRMT5 expression suppressed the proliferation of A549 cells.

Flow cytometry analysis of the sub G-phase 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 (Figure 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%) (Figure 3B, lower panel) was significantly higher than the proportion of NT shRNA-expressing cells in the G1-phase (51.103%) (Figure 3B, upper panel). 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 down-regulated and expression of the FGF17 and FGFBP1 genes was up-regulated in PRMT5 shRNA-expressing A549 cells compared with NT shRNA-expressing A549 cells (Figure 4A). Western blotting 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 compared with lane 1, and Figure 4C, lower panels compared with the upper 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...
Figure 4  Silencing PRMT5 expression decreased FGFR3 expression

(A) Expression of FGFRs and negative regulators of FGFR signalling (SPRY1, SPRY2, SPRY4, FLRT3 and Nedd4) in A549 cells expressing NT shRNA or PRMT5 shRNA. Relative expression is the 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 (upper panels) or PRMT5 shRNA (lower panels). Original magnification is ×400 and the scale bar represents 10 μm.

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 tumours formed from A549 cells expressing NT shRNA, PRMT5 shRNA or PRMT5 shRNA plus FGFR3. Large macroscopically visible tumours were found in the lungs of the nine mice injected with NT shRNA-expressing A549 cells (Figure 6A, top panel). However, no tumours were detected in the lungs of the eight mice injected with PRMT5 shRNA-expressing A549 cells (Figure 6A, middle panel). We found small tumours in the lungs of five of the eight mice injected with PRMT5 shRNA plus FGFR3-expressing A549 cells (Figure 6A, bottom panel, indicated by black arrows). The average size (mean ± S.E.M.) of the lung tumours in the mice injected with NT shRNA-expressing A549 cells (13.3 ± 4.3 mm²) was much higher than that of the lung tumours in mice injected with PRMT5 shRNA plus FGFR3-expressing A549 cells (2.6 ± 1.4 mm²; Figure 6B). These results indicate that PRMT5 is essential for growth of lung tumour xenografts, and that the growth occurs partially through the FGFR3 signalling.

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 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
Figure 6 Loss of PRMT5 expression abolished growth of lung tumour xenografts

(A) Lungs derived from mice injected with A549 cells stably expressing NT shRNA (top panel), PRMT5 shRNA (middle panel) or PRMT5 shRNA plus FGFR3 (bottom panel). Small tumours in lungs injected with A549 cells stably expressing PRMT5 shRNA plus FGFR3 (bottom panel) are indicated by black arrows. (B) Mean size of tumours in mouse lungs injected with A549 cells stably expressing NT shRNA, PRMT5 shRNA or PRMT5 shRNA plus FGFR3. Error bars indicate S.E.M.

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 [22]. 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 signalling 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 signalling in lung cancer might occur through up-regulation of transcription of genes of FGFs or FGFRs. In the present 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 signalling [SPRY (Sprouty homologue) 2, SPRY4 and FLRT3 (fibronectin leucine-rich transmembrane protein 3)] [43,44] was also increased in the PRMT5 shRNA-expressing A549 cells (Figure 4A). Furthermore, expression of Nedd4 (neuronal precursor cell-expressed developmentally down-regulated 4) more than doubled in the PRMT5 shRNA-expressing A549 cells (Figure 4A). Nedd4 is an E3 ubiquitin ligase that suppresses FGFR signalling via the ubiquitin-mediated degradation pathway [45]. Decreased SPRY and FLRT3 expression and increased Nedd4 expression might lead to significantly decreased FGFR signalling 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 signalling regulation is further supported by our finding that ectopic expression of FGFR3 partially restored the growth defect of A549 cells and tumour 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 signalling 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 present 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.

AUTHOR CONTRIBUTION

Fahao Zhang performed orthotopic injection of cells. Zhongping Gu and Shen Gao performed the other experiments. Zhiqiang Wang, Wencai Ma and Richard Davis performed the DNA microarray analysis. Zhengxin Wang wrote the paper.

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