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# *ANKRD18A* as a novel epigenetic regulation gene in lung cancer

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ABSTRACT

Lung cancer is one of the most common causes of cancer-related mortality worldwide. Effective early diagnosis and targeted therapies for lung cancer to reduce incidence and mortality would benefit from a better understanding of the key molecular changes that occur from normal to malignant tumor cells during lung cancer initiation and development, but these are largely unknown. Previous studies have shown that DNA methylation, an important mechanism for the regulation of gene expression, plays a key role in lung carcinogenesis. In this study, we screened a novel methylation gene, *ANKRD18A*, encoding ankyrin repeat domain 18A, to determine whether it is regulated by DNA methylation in lung cancer. Methylation-specific PCR and bisulfite sequencing PCR were used to analyze gene methylation status, and real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) examined mRNA levels. Promoter hypermethylation of *ANKRD18A* was detected in 68.4% (26/38) of lung cancer tissues but not (0/20) in normal lung tissues ($P < 0.01$), whereas *ANKRD18A* mRNA expression was significantly decreased in lung cancer tissues compared with adjacent normal tissues. In addition, we found that *ANKRD18A* expression was significantly decreased in 9 of 10 lung cancer cell lines. This was associated with hypermethylation of the *ANKRD18A* promoter region. Moreover, weak expression of *ANKRD18A* in methylated lung cancer cell lines increased markedly after treatment with the DNA methylation inhibitor 5-aza-2′-deoxycytidine. These results suggest that *ANKRD18A* hypermethylation and consequent mRNA alterations might be a vital molecular mechanism in lung cancer.



## 1. Introduction

The incidence and mortality of lung cancer have shown a rising trend and appear to have stabilized the leading cause of cancer-related mortality among men in China, Western Europe and the United States [1]. Although lung cancer can be treated by surgery, radiation, drugs and other means, the 5-year survival rate remains under 15%, mainly because of the lack of effective early diagnosis. Thus, an in-depth study on the molecular mechanisms of lung cancer may help to find novel and effective molecular biomarkers for early screening of high-risk groups to reduce lung cancer incidence and mortality. However, the molecular events responsible for its initiation and development are still largely unknown.

Emerging evidence indicates that epigenetic modification, especially DNA methylation, plays an important role in the development of lung carcinogenesis [2,3]. DNA methylation is considered to be the third mechanism of tumor suppressor gene inactivation and tumorigenesis following the loss of heterozygosity and mutations [4]. In addition to the methylation analysis of known tumor-associated genes, a growing body of studies has applied high-throughput screening technology to identify novel genes regulated by DNA methylation in many types of cancer [5–7]. Moreover, the functions of these genes are closely related to tumor cell growth, apoptosis and migration [5–7]. The identification of such genes may further our understanding of molecular mechanisms, and aid in the development of alternative approaches for diagnostic and therapeutic evaluation.

Through methylation-sensitive arbitrarily primed PCR, we have identified a novel preferentially methylated gene, ankyrin repeat domain 18A (*ANKRD18A*), in human lung cancer. *ANKRD18A* is located on chromosome 9p13.1, contains 16 exons [8,9], and its

*Abbreviations:* 5-aza-dC, 5-aza-2′-deoxycytidine; *ANKRD18A*, ankyrin repeat domain 18A; BSP, bisulfite sequencing polymerase chain reaction; MSP, methylation-specific polymerase chain reaction; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction.

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encoded protein sequence has a typical encoding domain with an ankyrin repeat. These repeat sequences usually contain 33 residues, and are commonly found in a protein sequence motif; they participate in eukaryotic, prokaryotic and viral protein–protein interactions in cell growth, cell cycle regulation, signal transduction and other functions closely related to a variety of diseases [10]. Previous studies have shown that genes encoding proteins with ankyrin repeats, such as *p16*, kidney ankyrin repeat-containing protein (*Kank*), *HACE1*, *ASPP1* and *ASPP2*, are often inactivated by methylation in many types of cancers [11–14]. However, limited research has been carried out on the regulation of *ANKRD18A* expression. In this study, we compared the methylation status of the *ANKRD18A* promoter region and its expression levels in normal and tumor tissue and cell lines. We also performed a demethylation experiment to identify the biological function of *ANKRD18A* methylation.

## 2. Materials and methods

### 2.1. Tissue samples and cell lines

After surgical resection, fresh lung cancer and normal lung tissues from patients were collected at the Affiliated Xi'nan Hospital of Third Military Medical University and immediately snap-frozen in liquid nitrogen, then kept at −80 °C before use. All experimental protocols were approved by the human ethics committee of the Medical Center of Third Military Medical University and written informed consent was obtained from all subjects.

The immortalized human bronchial epithelial cell line HBE and 10 lung cancer cell lines (A549, SPC-A-1, H1975, H358, H1650, LTEP, and H1395 (adenocarcinoma), H446 (small cell lung carcinoma), H460 (large cell carcinoma), 95D (highly metastatic giant cell carcinoma)) were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and the Cell Biology Institute of Chinese Academy of Science (Shanghai, China). Cell lines were cultured in RPMI 1640 medium (Gibco BRL, Rockville, MD) supplemented with 10% fetal bovine serum (Gibco BRL) at 37 °C in a humid incubator with 5% $CO_2$.

### 2.2. DNA extraction and methylation analysis

Total genomic DNA was isolated from normal and lung cancer tissues and cell lines using the DNA extraction kit (Promega, Madison, WI) according to the manufacturer's instructions. Genomic DNA was then modified using the EZ DNA Methylation-Gold Kit (Zymo Research, Orange, CA) following the manufacturer's protocol.

The primer sequences used for methylation-specific polymerase chain reaction (MSP) and bisulfite sequencing PCR (BSP) are shown in Table 1. MSP and BSP conditions for *ANKRD18A* have been standardized in our laboratory and previously reported [15,16]. For MSP, PCR were performed using a primer-specific annealing temperature (Table 1) for unmethylated and methylated reactions. Normal untreated or *Sss*I-methylase-treated (New England Biolabs, Beverly, MA) genomic DNA samples from blood provided fully unmethylated and fully methylated positive MSP controls and were used in all PCR experiments. Sterile water with no DNA template was included as a negative control. Following amplification, each PCR product was electrophoresed on a 3% agarose gel, stained with ethidium bromide and visualized under UV illumination. The investigators who performed the assays were blinded to all sample information. For BSP, amplified products were purified and cloned into the pGEM-T vector (Promega). Ten colonies of each specimen were randomly chosen for plasmid DNA extraction with the Promega Spin Mini kit (Promega), and were sequenced by an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) to identify the CpG methylation status.

**Table 1**
Primer sequences used in this study.

| Gene | Primer sequence (5′–3′) | Length (bp), position | Annealing temperature (°C) |
|---|---|---|---|
| *For MSP* | | | |
| ANKRD18A(M) | Forward: TTAGGGAGGGTAATATTTACGAGAC<br>Reverse: ATAAAAAACGACCTACTAACACGAC | 101<br>−1744 to −1644 | 56 |
| ANKRD18A(U) | Forward: TTTTAGGGAGGGTAATATTTATGAGAT<br>Reverse: ATAAAAAACAACCTACTAACACAAC | 103<br>−1746 to −1644 | 54 |
| *For BSP* | | | |
| ANKRD18A | Forward: GGGATTTTGAGTTTTGTATG<br>Reverse: AACCTCCTACACCTATCATCCTAAC | 280<br>−1815 to −1536 | 57 |
| *For RT-PCR* | | | |
| ANKRD18A | Forward: GAGGCTTGTGCCATCGTT<br>Reverse: AAGCAGGAGGGTGACGAT | 321<br>418 to 738 | 52 |
| β-actin | Forward: GGCATGGAGTCCTGTGG<br>Reverse: AGAAGCATTTGCGGTGG | 325<br>886 to 1210 | 58 |
| *For qRT-PCR* | | | |
| ANKRD18A | Forward: AAGCAAGAAAGGCTCCAAAGAAGT<br>Reverse: GAGCATGGCAATGTCTTTCTTCA | 117<br>973 to 1089 | 60 |
| β-actin | Forward: CCACGAAACTACCTTCAACTCC<br>Reverse: GTGATCTCCTTCTGCATCCTGT | 132<br>906 to 1037 | 60 |

### 2.3. RNA extraction and quantitative reverse transcription-PCR (qRT-PCR)

Total RNA was extracted from normal and lung cancer tissues and cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. cDNA was synthesized from 1 μg of total RNA using the PrimeScript® RT reagent Kit with gDNA Eraser (Takara, Shiga, Japan). RT-PCR and real-time qRT-PCR analyses were performed using primers and annealing temperatures shown in Table 1. The housekeeping gene β-actin was amplified as an internal control. Negative controls (distilled water) were also run for each sample. RT-PCR products were analyzed using 2% agarose gel electrophoresis stained with ethidium bromide. Real-time qRT-PCR detection was carried out using an iQ5 real-time detection system (Bio-Rad Laboratories, Hercules, CA) and SYBR® Premix Ex Taq™ II (Takara). *ANKRD18A* mRNA levels were normalized to β-actin and the $2^{-\Delta\Delta Ct}$ method was used to analyze the relative levels of *ANKRD18A* expression.

### 2.4. Treatment of lung cancer cell lines with 5-aza-2′-deoxycytidine (5-aza-dC)

To assess whether the mRNA expression of *ANKRD18A* was restored by 5-aza-dC treatment, demethylation experiments were performed as previously described [15,16]. Briefly, lung cancer cells with *ANKRD18A* hypermethylation were exposed to 10 μM of 5-aza-dC (Sigma, St Louis, MO) for three days, with cell media and drugs being replaced daily. The control culture was treated

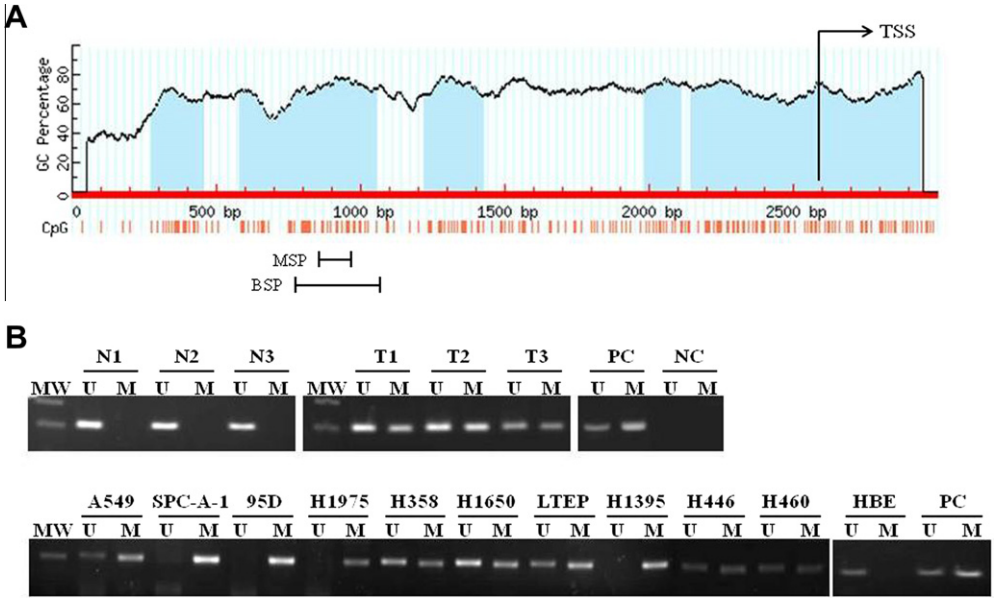


**Fig. 1.** (A) Diagram of the location of CpG islands in the promoter region of *ANKRD18A* with CpG sites indicated by vertical orange lines. MSP and BSP regions are indicated. TSS, transcription start site. (B) Representative electrophoresis results of MSP products from normal and tumor tissues and cell lines. Each lane contains products amplified from separate PCR reactions using specific primers for unmethylated (U) or methylated (M) DNA templates. MW, molecular weight; N, normal lung tissue; T, lung cancer tissue; PC, positive control, including fully unmethylated control (normal blood genomic DNA sample) and fully methylated control (normal blood genomic DNA sample treated with *Sss*I-methylase); NC, negative control (sterile water without the addition of DNA). *ANKRD18A* was unmethylated in all normal lung tissues and human bronchial epithelial cell line HBE but methylated in some tumor tissues and all lung cancer cell lines.

in parallel with DMSO at each time point. DNA and RNA were harvested on day three after initial treatment. The methylation status and mRNA expression of *ANKRD18A* were detected by MSP, RT-PCR and qRT-PCR (as described above). The primer sequences and annealing temperature used for PCR are shown in Table 1.

### 2.5. Statistical analysis

SPSS 16.0 software (SPSS, Inc., Chicago, IL) was used to analyze data. Differences in methylation frequency and mRNA expression between groups were estimated by the Chi-square test and the Student's *t*-test. All *P* values are 2-sided, and *P* values less than 0.05 were taken as statistically significant.

## 3. Results

### 3.1. Methylation of ANKRD18A gene in normal and tumor tissues and cell lines

Previously, we analyzed the aberrant methylation status of human lung cancer tissues through genome-wide methylation screening and found one hypermethylated DNA fragment in the *ANKRD18A* promoter region. CpG island prediction software analysis found that this area has a structure typical of CpG islands (Fig. 1A). To test whether *ANKRD18A* methylation was tumor-specific, we analyzed its methylation status in lung tumor ($n = 38$) and normal ($n = 20$) samples using MSP. Representative results are shown in Fig. 1B, and *ANKRD18A* was found to be unmethylated in all normal lung tissues but methylated in some tumor tissues. Further analysis showed that the extent of gene hypermethylation in human lung tumor tissue was 68.4% (26/38), while no normal lung tissue was methylated (0/20 = 0%). *ANKRD18A* was next found to be unmethylated in normal human bronchial epithelial cell line HBE but methylated in all of 10 lung cancer cell lines, being fully methylated in SPC-A-1, 95D, H1975 and H1395, and partially methylated in A549, H358, H1650, LTEP, H446 and H460, reflecting an allele-specific event (Fig. 1B).

We further validated the MSP results by BSP (Fig. 2A). Amplified fragments were cloned and 10 clones were sequenced for each amplification product from each subject. Fig. 2A shows the average methylation of each of the 29 CG pairs within the promoter CpG island region of *ANKRD18A*. The BSP results were consistent with those of MSP, in which dense methylation was found in methylated cell lines and tumor tissues but not in unmethylated normal lung tissues. Fig. 2B shows the same sequence analysis of genomic DNA extracted from lung cell line SPC-A-1 and a normal lung tissue sample after sodium bisulfite conversion. These findings suggest that *ANKRD18A* methylation is tumor-specific.

### 3.2. ANKRD18A gene expression downregulation is associated with promoter hypermethylation in lung cancer cell lines and tumor tissues

To determine whether *ANKRD18A* promoter methylation regulated its mRNA expression, we examined the mRNA expression of *ANKRD18A* in 10 pairs of lung cancer tissue and corresponding adjacent normal tissues and 10 lung cancer cell lines. Representative RT-PCR images are depicted in Fig. 3A. We found that the *ANKRD18A* mRNA level relative to β-actin in the methylated lung cancer sample was significantly lower than that in the matched unmethylated normal lung tissue sample.

Fig. 3B shows the detection of *ANKRD18A* mRNA expression in the normal human bronchial epithelial cell line HBE and 10 lung cancer cell lines. We found that the *ANKRD18A* transcript was nearly silenced in SPC-A-1, 95D, H1975, and H446 cells, and was reduced in A549, H358, H1650, LTEP, and H460 cell lines, all of which showed *ANKRD18A* hypermethylation. By contrast, *ANKRD18A* expression was readily detected in unmethylated HBE cells, suggesting aberrant gene downregulation of *ANKRD18A* in lung cancers. To our knowledge, this is the first determination of relative *ANKRD18A* mRNA levels in lung cancer cell lines and tissues by RT-PCR.

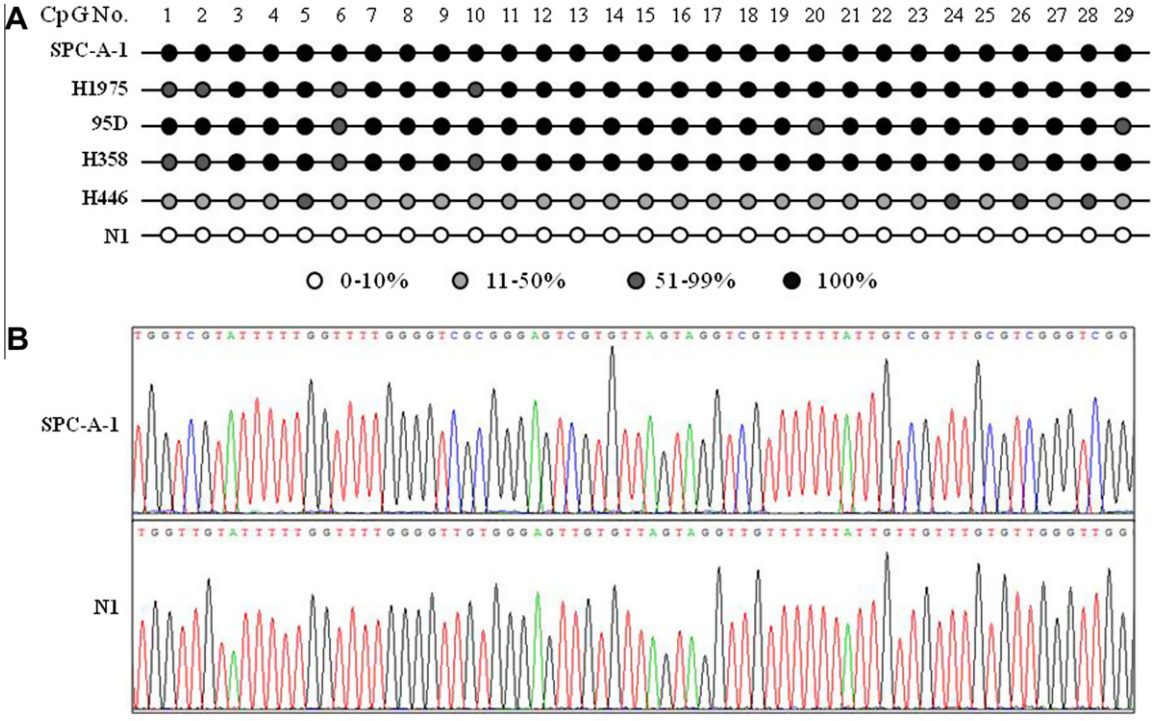


**Fig. 2.** Methylation analysis of *ANKRD18A* by bisulfite sequencing PCR (BSP). (A) Methylation status of the *ANKRD18A* promoter region confirmed by BSP. (B) Representative sequence analysis of genomic DNA extracted from lung cancer cell lines and normal tissue samples after sodium bisulfite conversion. CpG dinucleotide loci in normal tissue sample were unmethylated while all CpG loci were methylated in SPC-A-1 cells.

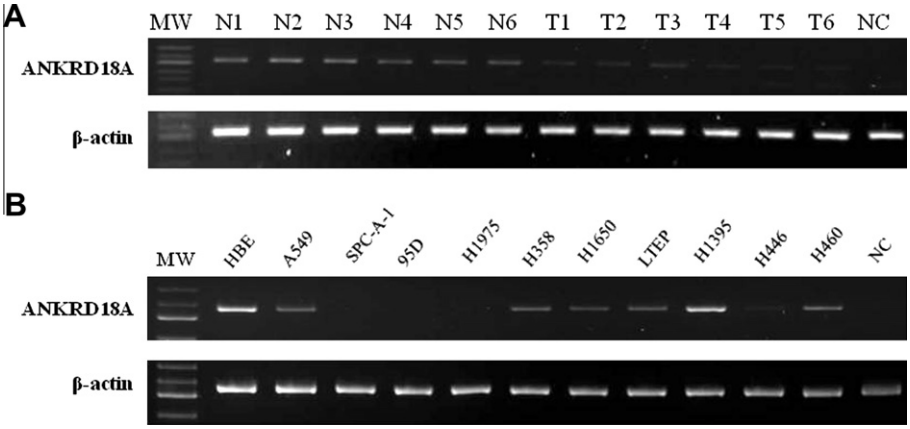


**Fig. 3.** Representative expression analysis of *ANKRD18A* mRNA in tissues and cell lines by RT-PCR. (A) *ANKRD18A* was highly expressed in normal lung tissues, but was significantly downregulated in corresponding lung cancer tissue samples. T1, T4 and T5: adenocarcinoma; T2, T3 and T6: squamous cell carcinoma. (B) *ANKRD18A* was downregulated or silenced in nine of ten lung cancer cell lines compared with the normal human bronchial epithelial cell line HBE.

### *3.3. Demethylation treatment with 5-aza-dC restored ANKRD18A expression*

To further confirm that promoter methylation is associated with inhibition of *ANKRD18A* expression, we measured *ANKRD18A* mRNA levels in *ANKRD18A*-hypermethylated lung cancer cell lines incubated with or without the DNA methylation inhibitor 5-aza-dC, which inhibits DNA methyltransferase activity. Methylated alleles were undetectable after 5-aza-dC treatment. As expected, the lung cancer cell lines 95D, SPC-A-1, H1975, and H446, which initially showed high levels of *ANKRD18A* methylation and loss of mRNA expression, were induced to express *ANKRD18A* following 5-aza-dC treatment (Fig. 4A). qRT-PCR confirmed these results, showing that endogenous *ANKRD18A* mRNA levels were significantly higher in the 5-aza-dC-treated group than in control cells (5-aza-dC *vs* DMSO, A549: 5.83 ± 0.68, SPC-A-1: 2.27 ± 0.03, 95D: 4.58 ± 0.42, H1975: 2.90 ± 0.35, H358: 3.67 ± 0.60, H446: 3.41 ± 0.61) (Fig. 4B). The changes in methylation status and expression strongly suggest that promoter hypermethylation directly contributes to the defective expression of *ANKRD18A*.

## 4. Discussion

Increasing evidence supports the hypothesis that tumor suppressor gene inactivation by epigenetic alterations is associated with lung carcinogenesis [3,4]. Previously, we applied genome-wide methylation analysis to screen and clarify several novel hypo-

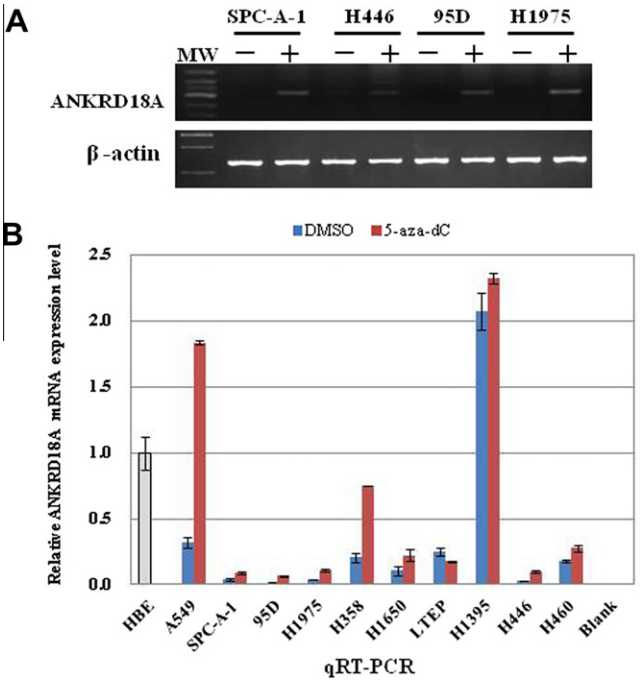


**Fig. 4.** Demethylation experiment of lung cancer cells with *ANKRD18A* hypermethylation exposed to 10 μM 5-aza-dC for three days. (A) *ANKRD18A* mRNA expression analysis by RT-PCR. (B) The expression of *ANKRD18A* mRNA transcripts significantly increased in lung cancer cells after 5-aza-dC treatment as shown by qRT-PCR. Data are presented as mean values relative to the normal human bronchial epithelial cell line HBE of three independent experiments.

and hypermethylated sites in rat lung cancer [15]. In this study, we demonstrated that downregulation of *ANKRD18A* expression is associated with aberrant promoter hypermethylation in lung cancer, suggesting that *ANKRD18A* is a novel epigenetic regulation gene. Indeed, we found that the *ANKRD18A* promoter is frequently hypermethylated in a cancer-specific manner in lung cancer cell lines and human tissues.

To our knowledge, this is the first study of *ANKRD18A* methylation in lung cancer. Notably, in all cases where *ANKRD18A* was hypermethylated in tumor tissue, aberrant methylation was also observed in the sputum and/or plasma specimens of patients, whereas *ANKRD18A* hypermethylation was undetected in samples from patients whose tumors lacked this epigenetic alteration (data not shown). These findings suggest that *ANKRD18A* methylation is a potential candidate for lung cancer molecular markers, although our previous results indicate that detection of p16 hypermethylation in both sputum and plasma could prove a more sensitive and specific method for the molecular diagnosis of lung cancer [11]. Future large prospective screening studies should validate sputum and plasma data, and it is conceivable that *ANKRD18A* methylation could be combined with other methylation markers to enhance sensitivity and/or specificity.

Epigenetic silencing of cancer-related genes has been shown to be reversible [17–19]. Consequently, we analyzed *ANKRD18A* expression in lung cancer cells cultured in the presence of the demethylation agent 5-aza-dC. We found that the defective expression of *ANKRD18A* in methylated lung cancer cell lines was markedly increased after 5-aza-dC treatment. Collectively, our data reveal that the epigenetic regulation mechanism (CpG island methylation) of *ANKRD18A* has an important role in its transcriptional downregulation, which may be responsible for the initiation and development of lung cancer.

The ANKRD family has also been investigated in association with neoplasia [20–22]. ANKRD1 is expressed in the majority of ovarian adenocarcinomas and higher tumor levels of ANKRD1 are associated with patients with worse outcomes. Moreover, *ANKRD1* mRNA levels are correlated with platinum sensitivity in cell lines [20]. ANKRD 13A, 13B, and 13D proteins, binding to the Lys-63-linked polyubiquitin moiety of epidermal growth factor receptor (EGFR) at the plasma membrane, regulate the rapid internalization of ligand-activated EGFR [21]. ANKRD26 causes obesity and diabetes in mice and increases spontaneous and induced adipogenesis in mouse embryonic fibroblasts [23]. Recently, it was found that interaction of the tumor suppressor RARRES1 with ANKRD26-like family member 1A regulates microtubule function [22]. These findings suggest that the ANKRD family plays an important role in carcinogenesis. Therefore, further research into the biological function of *ANKRD18A* in lung carcinogenesis would greatly improve our understanding of the molecular mechanisms of lung cancer.

In conclusion, this is the first study to describe *ANKRD18A* as a novel methylation gene in lung cancer, thereby providing a powerful tool for early diagnosis and a potential target for treatment. Moreover, our findings indicate that the hypermethylation of promoter regulatory elements contributes to lower *ANKRD18A* expression in lung cancer, which may lead to disease pathogenesis. Together, our results provide novel insights into the pathogenesis of lung cancer, and assist our understanding of the involvement of epigenetic factors in this disease.

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## References

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2012, CA: Cancer J. Clin. 62 (2012) 10–29.
[2] R. Feil, M.F. Fraga, Epigenetics and the environment: emerging patterns and implications, Nat. Rev. Genet. 13 (2012) 97–109.
[3] M. Rodríguez-Paredes, M. Esteller, Cancer epigenetics reaches mainstream oncology, Nat. Med. 17 (2011) 330–339.
[4] S. Sharma, T.K. Kelly, P.A. Jones, Epigenetics in cancer, Carcinogenesis 31 (2010) 27–36.
[5] L. Xu, X. Li, E.S. Chu, G. Zhao, M.Y. Go, Q. Tao, H. Jin, Z. Zeng, J.J. Sung, J. Yu, Epigenetic inactivation of BCL6B, a novel functional tumour suppressor for gastric cancer, is associated with poor survival of gastric cancer, Gut 61 (2012) 977–985.
[6] W. Liu, X. Li, E.S. Chu, M.Y. Go, L. Xu, G. Zhao, L. Li, N. Dai, J. Si, Q. Tao, J.J. Sung, J. Yu, Paired box gene 5 is a novel tumor suppressor in hepatocellular carcinoma through interaction with p53 signaling pathway, Hepatology 53 (2011) 843–853.
[7] K.F. Cheung, C.N. Lam, K. Wu, E.K. Ng, W.W. Chong, A.S. Cheng, K.F. To, D. Fan, J.J. Sung, J. Yu, Characterization of the gene structure, functional significance, and clinical application of RNF180, a novel gene in gastric cancer, Cancer 118 (2012) 947–959.
[8] K. Kimura, A. Wakamatsu, Y. Suzuki, T. Ota, T. Nishikawa, R. Yamashita, J. Yamamoto, M. Sekine, K. Tsuritani, H. Wakaguri, S. Ishii, T. Sugiyama, K. Saito, Y. Isono, R. Irie, N. Kushida, T. Yoneyama, R. Otsuka, K. Kanda, T. Yokoi, H. Kondo, M. Wagatsuma, K. Murakawa, S. Ishida, T. Ishibashi, A. Takahashi-Fujii, T. Tanase, K. Nagai, H. Kikuchi, K. Nakai, T. Isogai, S. Sugano, Diversification of transcriptional modulation: large-scale identification and characterization of putative alternative promoters of human genes, Genome Res. 16 (2006) 55–65.
[9] R.L. Strausberg, E.A. Feingold, L.H. Grouse, J.G. Derge, R.D. Klausner, F.S. Collins, L. Wagner, C.M. Shenmen, G.D. Schuler, S.F. Altschul, B. Zeeberg, K.H. Buetow, C.F. Schaefer, N.K. Bhat, R.F. Hopkins, H. Jordan, T. Moore, S.I. Max, J. Wang, F. Hsieh, L. Diatchenko, K. Marusina, A.A. Farmer, G.M. Rubin, L. Hong, M. Stapleton, M.B. Soares, M.F. Bonaldo, T.L. Casavant, T.E. Scheetz, M.J. Brownstein, T.B. Usdin, S. Toshiyuki, P. Carninci, C. Prange, S.S. Raha, N.A. Loquellano, G.J. Peters, R.D. Abramson, S.J. Mullahy, S.A. Bosak, P.J. McEwan, K.J. McKernan, J.A. Malek, P.H. Gunaratne, S. Richards, K.C. Worley, S. Hale, A.M. Garcia, L.J. Gay, S.W. Hulyk, D.K. Villalon, D.M. Muzny, E.J. Sodergren, X. Lu, R.A. Gibbs, J. Fahey, E. Helton, M. Ketteman, A. Madan, S. Rodrigues, A. Sanchez, M. Whiting, A. Madan, A.C. Young, Y. Shevchenko, G.G. Bouffard, R.W. Blakesley, J.W. Touchman, E.D. Green, M.C. Dickson, A.C. Rodriguez, J. Grimwood, J. Schmutz, R.M. Myers, Y.S. Butterfield, M.I. Krzywinski, U. Skalska, D.E. Smailus, A. Schnerch, J.E. Schein, S.J. Jones, M.A. Marra, Mammalian gene collection program team., generation and initial analysis of more than 15,000 full-length

human and mouse cDNA sequences, Proc. Natl. Acad. Sci. USA 99 (2002) 16899–16903.
[10] S.G. Sedgwick, S.J. Smerdon, The ankyrin repeat: a diversity of interactions on a common structural framework, Trends Biochem. Sci. 24 (1999) 311–316.
[11] Y. Liu, Q. An, L. Li, D. Zhang, J. Huang, X. Feng, S. Cheng, Y. Gao, Hypermethylation of p16INK4a in Chinese lung cancer patients: biological and clinical implications, Carcinogenesis 24 (2003) 1897–1901.
[12] J. Zhao, G. Wu, F. Bu, B. Lu, A. Liang, L. Cao, X. Tong, X. Lu, M. Wu, Y. Guo, Epigenetic silence of ankyrin-repeat-containing, SH3-domain-containing, and proline-rich-region- containing protein 1 (ASPP1) and ASPP2 genes promotes tumor growth in hepatitis B virus-positive hepatocellular carcinoma, Hepatology 51 (2010) 142–153.
[13] S. Sarkar, B.C. Roy, N. Hatano, T. Aoyagi, K. Gohji, R. Kiyama, A novel ankyrin repeat-containing gene (Kank) located at 9p24 is a growth suppressor of renal cell carcinoma, J. Biol. Chem. 277 (2002) 36585–36591.
[14] L. Zhang, M.S. Anglesio, M. O'Sullivan, F. Zhang, G. Yang, R. Sarao, P.N. Mai, S. Cronin, H. Hara, N. Melnyk, L. Li, T. Wada, P.P. Liu, J. Farrar, R.J. Arceci, P.H. Sorensen, J.M. Penninger, The E3 ligase HACE1 is a critical chromosome 6q21 tumor suppressor involved in multiple cancers, Nat. Med. 13 (2007) 1060–1069.
[15] W.B. Liu, J.Y. Liu, L. Ao, Z.Y. Zhou, Y.H. Zhou, Z.H. Cui, H. Yang, J. Cao, Dynamic changes in DNA methylation during multistep rat lung carcinogenesis induced by 3-methylcholanthrene and diethylnitrosamine, Toxicol. Lett. 189 (2009) 5–13.
[16] W.B. Liu, Z.H. Cui, L. Ao, Z.Y. Zhou, Y.H. Zhou, X.Y. Yuan, Y.L. Xiang, J.Y. Liu, J. Cao, Aberrant methylation accounts for cell adhesion-related gene silencing during 3-methylcholanthrene and diethylnitrosamine induced multistep rat lung carcinogenesis associated with overexpression of DNA methyltransferases 1 and 3a, Toxicol. Appl. Pharmacol. 251 (2011) 70–78.
[17] X. Li, K.F. Cheung, X. Ma, L. Tian, J. Zhao, M.Y. Go, B. Shen, A.S. Cheng, J. Ying, Q. Tao, J.J. Sung, H.F. Kung, J. Yu, Epigenetic inactivation of paired box gene 5, a novel tumor suppressor gene, through direct upregulation of p53 is associated with prognosis in gastric cancer patients, Oncogene 31 (2012) 3419–3430.
[18] J. Yu, X. Ma, K.F. Cheung, X. Li, L. Tian, S. Wang, C.W. Wu, W.K. Wu, M. He, M. Wang, S.S. Ng, J.J. Sung, Epigenetic inactivation of T-box transcription factor 5, a novel tumor suppressor gene, is associated with colon cancer, Oncogene 29 (2010) 6464–6474.
[19] P.M. Das, A.D. Thor, S.M. Edgerton, S.K. Barry, D.F. Chen, F.E. Jones, Reactivation of epigenetically silenced HER4/ERBB4 results in apoptosis of breast tumor cells, Oncogene 29 (2010) 5214–5219.
[20] L.L. Scurr, A.D. Guminski, Y.E. Chiew, R.L. Balleine, R. Sharma, Y. Lei, K. Pryor, G.V. Wain, A. Brand, K. Byth, C. Kennedy, H. Rizos, P.R. Harnett, A. deFazio, Ankyrin repeat domain 1, ANKRD1, a novel determinant of cisplatin sensitivity expressed in ovarian cancer, Clin. Cancer Res. 14 (2008) 6924–6932.
[21] H. Tanno, T. Yamaguchi, E. Goto, S. Ishido, M. Komada, The Ankrd 13 family of UIM-bearing proteins regulates EGF receptor endocytosis from the plasma membrane, Mol. Biol. Cell 23 (2012) 1343–1353.
[22] Z.J. Sahab, M.D. Hall, Y. Me Sung, S. Dakshanamurthy, Y. Ji, D. Kumar, S.W. Byers, Tumor suppressor RARRES1 interacts with cytoplasmic carboxypeptidase AGBL2 to regulate the α-tubulin tyrosination cycle, Cancer Res. 71 (2011) 1219–1228.
[23] X.F. Liu, T.K. Bera, C. Kahue, T. Escobar, Z. Fei, G.A. Raciti, I. Pastan, ANKRD26 and its interacting partners TRIO, GPS2, HMMR and DIPA regulate adipogenesis in 3T3-L1 cells, PLoS ONE 7 (2012) e38130.