Histone methyltransferase SETD1A participates in lung cancer progression

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Research

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Abstract

Lung cancer is the leading cause of cancer-related death worldwide, with an estimated 1.2 million deaths each year. Despite advances in lung cancer treatment, 5-year survival rates are lower than ~15%, which is attributed to diagnosis limitations and current clinical drug resistance. Recently, more evidence has suggested that epigenome dysregulation is associated with the initiation and progress of cancer, and targeting epigenome-related molecules improves cancer symptoms. Interestingly, some groups reported that the level of methylation of histone 3 lysine 4 (H3K4me3) was increased in lung tumors and participated in abnormal transcriptional regulation. However, a mechanistic analysis is not available. In this report, we found that the SET domain containing 1A (SETD1A), the enzyme for H3K4me3, was elevated in lung cancer tissue compared to normal lung tissue. Knockdown of SETD1A in A549 and H1299 cells led to defects in cell proliferation and epithelial-mesenchymal transition (EMT), as evidenced by inhibited WNT and TGFβ pathways, compared with the control group. Xenograft assays also revealed a decreased tumor growth and EMT in the SETD1A silenced group compared with the control group. Mechanistic analysis suggested that SETD1A might regulate tumor progression via several critical oncogenes, which exhibited enhanced H3K4me3 levels around transcriptional start sites in lung cancer. This study illustrates the important role of SETD1A in lung cancer and provides a potential drug target for treatment.

Introduction

In the United States, a total of 228150 estimated new lung cancer cases (men 116,440 and women 111,710) will be diagnosed in 2019, which is the second highest incidence rate among all types of cancer; lung cancer causes 142,670 estimated deaths (men 76,650, women 66,020)\(^1\). This number is over 1.2 million globally\(^2\). Despite advances in research on lung cancer, one-quarter of cancer deaths are attributed to lung cancer, and the high mortality has been steady for more than 25 years\(^1\). In contrast to other cancers, over one-half of lung cancer patients were diagnosed at a distant stage with locally or widely metastatic tumors, resulting in a poor 5-year survival of ~ 15%\(^1,3\). In addition, current clinical drugs for lung cancer are often effective initially, with a later loss of function in the majority of patients, owing to drug resistance of the tumor cells\(^4\). Thus, it is urgent to identify novel biomarkers or molecular targets for early diagnosis and treatment\(^5\).

Recently, more evidence has suggested that epigenome dysregulation is associated with the initiation and progression of cancer\(^6\). Epigenetic mechanisms include DNA methylation, noncoding RNA regulation and histone modification, which mediates transcription of downstream genes by modification of nucleosomal histone proteins (H2A, H2B, H3, and H4), including acetylation, phosphorylation, ADP-ribosylation, biotinylation, ubiquitination, and methylation\(^7\). Indeed, recent studies demonstrated that aberrant histone methylation was often present in carcinoma and played an important role in tumorigenesis. For example, a genome-wide study indicated that histone 3 lysine 27 methylation modification (H3K27me3) is more frequent in human cancer cells, including colon, breast, and prostate
cancer cell lines, than in normal cells. Enhanced H7K79me2/3 promoted the transcription of *HOXA9* and *MEIS1*, which are important drivers for leukemogenesis. In breast cancer cells, abnormal histone 4 arginine 3 methylation modification (H4R3me2a) of the *ZEB1* promoter region activated ZEB1 expression and enhanced the epithelial-mesenchymal transition (EMT) and cellular senescence in cancer. A meta-analysis suggested that the expression of histone 3 lysine 4 methylation (H3K4me) was associated with the prognosis of patients with cancers, including lung, colon, pancreas, esophagus, liver and cervical tumors. Interestingly, other research also indicated that an increased H4K4me3 level was present in lung adenocarcinoma cell lines and participated in abnormal transcriptional regulation. However, the mechanism is not clear.

In mammals, the methylation of H3K4 is mainly catalyzed by the SET1/KMT2 family of methyltransferase, while demethylation is performed by LSD1/2 and KDM5 family proteins. Recent evidence also demonstrated the close correlation between methyltransferase/demethylase and cancer processes. In this report, we found that SET domain containing 1A (SETD1A), a member of the SET1 family, was increased in lung tumors, and knockdown of SETD1A with shRNA led to defects in cell proliferation and EMT, as evidenced by Western blots of the related proteins. Xenograft experiments also suggested that SETD1A silencing in A549 and H1299 lung cancer cells prevented tumorigenesis and EMT in nude mice. Mechanistic analysis indicated that SETD1A might regulate tumor progression via several critical oncogenes, which exhibited enhanced H3K4me3 levels around transcriptional start sites in lung cancer.

**Results**

**SETD1A participates in lung cancer progression in clinical cases**

It was reported that SETD1A was associated with tumor progression in breast and colorectal cancer. We analyzed changes in the copy numbers of the SETD1A gene among different cancers in the TCGA database (7460 samples/7421 patients) and found that the amplification mutation was more frequent in non-small cell lung cancer (NSCLC) than any other cancer except breast cancer (FIGURE S1). Further analysis suggested that the SETD1A mRNA level was significantly elevated in samples, as either gain or amplification in copy number of SETD1A, compared to samples with shallow deletion or diploid mutations, which indicated that the amplification mutation of SETD1A loci led to upregulated SETD1A transcription in lung cancer (FIGURE 1A). The microarray data from other independent research groups also revealed that enhanced SETD1A mRNA was present in lung cancer tissue and gradually increased with the degree of malignancy of the lung tumors (FIGURE 1B-C). In addition, compared to small airway epithelial cells (SAEC), upregulated SETD1A was also found in nearly all lung adenocarcinoma cell lines (25/26) (FIGURE 1D). Moreover, Kaplan-Meier analysis on the OncoLnc website (data from TCGA) showed a high inverse correlation between the SETD1A level and survival rate of lung cancer patients (FIGURE 1E). The function of other components related to SETD1A methylation were also increased, and
these changes were partly correlated with overall survival status (FIGURE S2). These results suggested that SETD1A was associated with tumor progression in lung cancer.

**SETD1A promotes lung cancer cell proliferation and EMT**

To address the role of elevated SETD1A in lung cancer, we selected the A549 and H1299 cell lines for functional analysis, which exhibited modest and enhanced SETD1A overexpression, respectively (FIGURE 1D). We stably knocked down *SETD1A* in the human lung cancer cell lines A549 and H1299 with lentivirus-encoded shRNAs (FIGURE 2A-B). The cell viability assay revealed that *SETD1A* silencing in A549 or H1299 led to a quick and significant inhibition of cell proliferation, as evidenced by a two-thirds reduction in cell number after 6 days in culture (FIGURE 2C and D). Because the WNT pathway plays a critical role in stimulating lung cancer cell cycle progression, we assessed activated β-catenin (Non-P-CTNNB1) and cell cycle protein PCNA and found a robust decrease in the *SETD1A* silenced group compared with the control group (FIGURE 2A, B, E and F). In addition, Western blotting of several important proteins associated with cell growth in lung cancer also revealed significant reductions in *SETD1A* knockdown cells, including decreases in AKT1 and phosphorylated STAT3 (P-STAT3) but not ERK signaling (P-MAPK1) (FIGURE 2E and F).

Migration ability is another important index of tumor severity. In light of the positive correlation between SETD1A expression and the degree of malignancy of lung cancer (FIGURE 1C), we first performed a wound healing assay to test directional motility of A549 cells. The results showed that the cells A549 lacking SETD1A moved more slowly compared with the control group (FIGURE 3A). Quantification analysis of wound width revealed that the migration ability was approximately 1.5- to 2-fold faster in control A549 cells compared to knockdown cells (FIGURE 3B). In addition, we also monitored cell migration with a transwell chamber experiment. Fewer A549 or H1299 cells passed through the porous membrane in the *SETD1A* knockdown group compared with the control group (FIGURE 3C-F), indicating that SETD1A could increase lung cancer cell migration. Next, the role of SETD1A in cell invasion was tested. Knockdown of *SETD1A* in A549 or H1299 significantly decreased the number of cells that passed though the Matrigel and porous supporting membrane, as evidenced by fewer cells stained with crystal violet (FIGURE 3C-F) compared with the control cells. In summary, these experiments indicated that SETD1A facilitated both cellular motility and invasiveness in lung cancer cells.

Lung cancer cells often undergo EMT and invade blood vessels, migrating to tissues including brain, bone or liver to form new metastases; thus, the EMT is the first step in lung tumor invasion and migration. It is reported that TGFβ signaling is crucial for EMT, so we measured molecules in the TGFβ pathway to determine the role of SETD1A in EMT. The upregulated CDH1 and reduced mesenchymal cell markers (CDH2, ZEB1, phosphorylated SMAD2 (P-SMAD2) and SNAI1) indicated that A549 and H1299 maintained their epithelial character in *SETD1A* silenced cells compared with the control group (FIGURE 3G and H). Thus, enhanced SETD1A expression in lung cancer accelerated the cell transition from epithelial-like to mesenchymal character.
SETD1A facilitates tumor growth and EMT \textit{in vivo}

To verify our results in cultured cancer cells, we performed a xenograft assay and monitored tumor growth. It is not surprising that \textit{SETD1A} silenced cells grew much more slowly than the control cells in tumor size and weight (A549, FIGURE 4A-C; H1299, FIGURE 4D-F). In addition, qPCR analysis of xenograft tumors also suggested that downregulation of \textit{SETD1A} resulted in a dramatic reduction in the expression of several critical oncogenes, such as tumor malignancy genes (\textit{MYC}, \textit{GLI1} and \textit{FOXM1}, \textit{STAT3} and \textit{FAK}), epigenetic modification enzymes (\textit{DNMT1}, \textit{SETDB1} and \textit{HDAC1}) and mesenchymal cell markers (\textit{CDH2, VIM, ZEB1 and SNAIL1}). Meanwhile, the expression of \textit{CDH1}, the epithelial character marker, sharply increased in \textit{SETD1A} silenced xenograft tumors (FIGURE 4G-J) compared with control tumors, which indicated that downregulation of \textit{SETD1A} inhibited EMT in xenograft tumors. In brief, reduced \textit{SETD1A} could prevent tumorigenesis and EMT of lung cancer.

\textbf{SETD1A regulates oncogene expression in lung cancer}

\textit{SETD1A} is an important methyltransferase to catalyze H3K4me3. Because more H3K4me3 was present in lung cancer cell lines\textsuperscript{12, 13}, we monitored the level of H3K4me3 in \textit{SETD1A} knockdown A549 cells. Immunofluorescence showed that \textit{SETD1A} silencing resulted in an obvious signal reduction of H3K4me3 in the global nucleus (FIGURE 5A-B). H3K4me3 modification could promote transcription of downstream genes\textsuperscript{27}; thus, the global decrease indicated expression changes in multiple genes. We analyzed CHIP-seq data in the ENCODE database (https://www.encodeproject.org/). Interestingly, enhanced H3K4me3 levels are frequently localized upstream of several oncogenes that are critical to the growth and metastasis of lung tumors (FIGURE S3-S4)\textsuperscript{28-33}. Indeed, Western blotting suggested that \textit{SETD1A} silencing reduced the expression of these oncogenes, including \textit{MYC}, \textit{GLI1}, \textit{FOXM1}, \textit{DNMT1}, \textit{SETDB1} and \textit{HDAC1} (FIGURE 5C and F), which indicated that \textit{SETD1A} might be a master gene to regulate lung cancer progression via H3K4me3 levels.

\textbf{Discussion}

It is reported that enhanced \textit{SETD1A} is associated with tumorigenesis and metastasis in colorectal and breast cancer\textsuperscript{21, 22}. Here, we found that \textit{SETD1A} was increased in lung cancer tissues compared with adjacent nontumor tissues and inversely correlated with overall survival status. Further research showed that \textit{STED1A} silencing resulted in defects in cancer growth and EMT \textit{in vitro} and \textit{in vivo}. Mechanistic analysis suggested that \textit{SETD1A} might regulate multiple critical oncogenes in lung cancer via global H3K4me3 levels.

The methylation and of H3K4 is performed by histone methylases (\textit{SETD1A/B} and \textit{KMT2} family)\textsuperscript{14-19}. Enhanced H3K4me3 was confirmed in lung cancer\textsuperscript{12, 13}, which was attributed to upregulated \textit{SETD1A}, owing to no differences in other histone methylases between lung cancer tissues and adjacent nontumor tissues (FIGURE S5). However, the downstream effects of \textit{SETD1A} are different in different tumors. In colorectal carcinoma, enhanced \textit{SETD1A} elevated H3K4me3 levels globally and activated multiple gene
transcription in WNT signaling(22), while upregulated SETD1A catalyzed H3K4me3 upstream of matrix metalloproteinase (MMP) genes and promoted breast cancer metastasis, although SETD1A silencing does not result in the downregulation of global H3K4me3(21). In lung cancer, we found that globally downregulated H3K4me3 and several critical oncogenes were reduced in SETD1A knockdown A549 cells. In addition, CHIP-seq analysis also showed enhanced H3K4me3 levels around transcriptional start sites in lung cancer cell lines. Thus, we speculated that SETD1A might regulate lung tumor growth and EMT via the transcriptional activation of multiple oncogenes that are involved in various pathways.

Several important gene mutants are confirmed in lung cancer and used as therapeutic targets for clinical treatment, such as EGFR (Gefitinib, Erlotinib, Afatinib, and Osimertinib), ALK (Crizotinib, ceritinib, alectinib, and brigatinib) and ROS1 (Crizotinib)(4). However, the curative effect is unsatisfactory owing to 5-year survival index of less than ~15% and the drug resistance of tumor cells(4). Thus, it is urgent to find new therapeutic strategies for lung cancer. Recently, some groups have focused on histone methylation. EPZ-5676, the inhibitor for DOT1L which is essential to H3K79 methylation, has completed a phase I clinical trial for leukemia (ClinicalTrials.gov identifier: NCT02141828). Enhancer of zeste homologue 2 (EZH2) is the catalytic component of the polycomb repressive complex 2 (PRC2) and regulates H3K27 modification; some inhibitors for EZH2 have been tested for cancer therapy in animals and have been tested in clinical trials (ClinicalTrials.gov identifier: NCT 02082977, NCT 01897571, NCT 02601937, NCT 02601950 and NCT 02395601). Our research suggested that SETD1A might be a master gene to drive the expression of several critical oncogenes in lung cancer. Thus, SETD1A could be a potential drug target for lung tumor treatment. At present, combination epigenetic therapy is widespread and our results provide a new candidate for combined treatment.

Materials And Methods

Cell lines

All cells were from Cell bank of Chinese Academy of Sciences. HEK 293T (SCSP-502) cells are grown in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, 11965118) containing 10% fetal calf serum (FBS; Fetal Bovine Serum) supplemented with penicillin (Sangon Biotech, A603460, 100 U/ mL) and streptomycin (Sangon Biotech A100382, 100 μg/ mL). A549 cells are cultured in 10%FBS-F12K medium (Sigma-Aldrich, N3520) supplemented with penicillin (Sangon Biotech, A603460, 100 U/ mL) and streptomycin (Sangon Biotech A100382, 100 μg/ mL). H1299 cells (SCSP-589) are grown in 10%FBS-RPMI 1640 Medium (Gibco, 11875093) with penicillin (Sangon Biotech, A603460, 100 U/ mL) and streptomycin (Sangon Biotech A100382, 100 μg/ mL). Cells were cultured in 37℃ incubator (Thermo Scientific, BB150) with a humidified atmosphere containing 5% CO₂.

Generation of SETD1A silenced cell line

HEK 293T cells were co-transfected with pLKO.1-puro cloning vector (addgene, #8453) encoding SETD1A-specific shRNA (GenScript, shSETD1A-
1: CCGCGGAAGAAGAAGCTCCGATTTCTCGAGAAATCGGAGCTTCTTCTTCCGTTTTTTG, shSETD1A-
2: CCGGCTTTGCGGAGAAGAAGCTGTACTCGAGTACAGCTTCTTCCGCAAAGTTTTTTG), Gal-pol and the
pCMV-VSV-G vector (Addgene, #8454) using polyethylenimine (PEI). 48h later, the supernatant of medium
was filtered by 0.45 μm filter, mixed with polybrene (10 μg/ mL) and infected A549/ H1299 cells. 48h
later, selected cells with 1 μg/ mL puromycin (Gibco, A1113803) and survival cells were harvested after 3
days.

**Western blot**

Cells were washed by PBS saline and lysed with RIPA buffer (150 mM NaCl, 2 mM EDTA (pH 8.0), 50 mM
Tris-HCl (pH 7.4), 0.1% SDS, 1% Nonidet P-40, 1 mM Na3VO4, 50 mM NaF and protease inhibitor cocktail
(Roche, 05892791001)). Protein concentration was measured by BCA Protein Assay Kit (Pierce, 23227)
and ~20 μg protein amount for each well of gel. Protein was separated by 10%SDS-PAGE gel (GenScript,
M00664) and electrotransferred to PVDF membrane (Millipore, IPVH00005). Next, the membrane was
blocked by blocking buffer with 5% non-fat milk in TBS and 0.1% Tween 20 (Sangon Biotech, A100777)
for 1h, and incubated overnight at 4℃ with primary antibody diluted in TBS and 0.1% Tween 20. Second
day, the membrane was incubated for 1h at room temperature with secondary antibody diluted in
blocking buffer (1:1000), combined with substrate (Tanon, #180-5001) and exposed with digital imager
(Tanon 4600). Image J software was used for immunoblot densitometric analysis. The primary
antibodies were β-Actin (Cell Signaling Technology, #3700. 1:10,000), SETDA1 (Cell Signaling Technology,
#61702. 1:1,000), SNAIL (Cell Signaling Technology, #3879. 1:1,000), p-SMAD2 (Cell Signaling
Technology, #18338. 1:1,000, phosphorylated at Ser465/467), SMAD2 (Cell Signaling Technology, #5339.
1:1,000), HDAC1 (Cell Signaling Technology, #5356. 1:2,000), DNMT1 (Cell Signaling Technology, #5032.
1:2,000), FOXM1 (Cell Signaling Technology, #5436. 1:2,000), p-STAT3 (Cell Signaling Technology,
#9145. 1:1,000, phosphorylated at Tyr405), STAT3 (Cell Signaling Technology, #9139. 1:2,000), PCNA
(Cell Signaling Technology, #13110. 1:3,000), AKT1 (Cell Signaling Technology, #75692. 1:2,000), p-
ERK1/2 (Cell Signaling Technology, #4370. 1:3,000, phosphorylated at Thr202/Tyr204), E-Cadherin (Cell
Signaling Technology, #3195. 1:1,000), N-Cadherin (Cell Signaling Technology, #13116.1:1,000), ZEB1
(Cell Signaling Technology, #3396. 1:1,000), GLI1 (Cell Signaling Technology, #3538. 1:1,000), c-Myc (Cell
Signaling Technology, #5605. 1:3,000), and SETDB1 (Cell Signaling Technology, #2196. 1:1,000).
Secondary antibodies conjugated with horseradish peroxidase (HRP) were from Thermo Fisher (31460
and 31430).

**Cell proliferation assay**

Cells were seeded at a density of 1×10^5 cells in one 10 cm dish. The number of viable cells was counted
every other day.

**Wound healing analysis**

The same number of A549 or H1299 cells was seeded in a 6 cm dish to ensure >90% density the next day.
Then, cells were wounded with a sterile pipette tip, and new culture medium was added. The images of
wound healing were randomly captured at the indicated time points (24 h and 48 h). The speed of wound healing was quantified by the wound width. Leica DM2500 microscope (5×) and Image J software was used to analyzed wound width.

**Migration and invasion assay**

A Transwell chamber with a porous membrane (Corning, catalog no. 3422) was placed in a 24-well plate. Then, the same number ($1 \times 10^5$) of A549 or H1299 cells in 200 µl of serum-free culture medium was added to the upper chamber, and the lower chamber contained 500 µl of growth medium containing 10% FBS. After 12 h, nonmigrated cells on the top of the porous membrane were removed by a cotton swab, and the migrated A549 or H1299 cells on the bottom surface were fixed (4% paraformaldehyde) and stained with 0.5% crystal violet. The cells that passed though the membrane were visualized by the size of the colored area. Each experiment was repeated 3 times.

For the invasion assay, the Matrigel (Corning, catalog no. 354234) was diluted (1:8) with PBS and poured into chambers above which were placed in 24-well plates. Then, $1 \times 10^6$ cells were placed on the Matrigel, and the lower chamber contained 500 µl of growth medium containing 10% FBS. The cells were cultured at 37°C. Then, 24 h later, the cells that passed through the Matrigel and porous membrane were visualized with the same protocol as in the migration assay. Each experiment was repeated 3 times. OLYMPUS SZX10 microscope (8×) and Image J software was used to analyzed cell number.

**Mouse xenograft models**

Six-week-old male nude mice were purchased from The Laboratory Animal Center (Shandong University, Jinan, China) and randomly divided into two groups. Then, $5 \times 10^5$ A549 or H1299 cells in 100 µl PBS were injected subcutaneously in the back of each mouse, and the tumor size was calculated as length×width$^2$/2 at the indicated day. At the end point (A549, 35 days; H1299, 25 days), the tumors were collected for weighing and RNA expression analysis. The mice were housed in SPF condition with 12h daytime and 12h night. All experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of the Shandong University.

**Immunofluorescence**

Cells were washed with phosphate buffered saline (PBS) three times and fixed with 4% PFA in PBS for 15 min, then cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min, blocked with 0.2% fish skin gelatin (Sigma-Aldrich, #G7041) in PBS for 30 min at room temperature and incubated with primary antibody diluted in PBS at 4°Covernight. Next day, second antibody diluted in PBS was used to combine primary antibody at room temperature for 1h and the location was detected with ZEISS LMF880 laser scanning confocal microscope. The primary antibodies were SET1A (CST, #61702. 1:400) and H3K4me3 (abcam, #ab1012.1:200). The secondary antibody was Cy3-conjugated anti-rabbit IgG (Sigma-Aldrich, #C2306. 1:1,000). Cell nuclei were counterstained with DAPI.
Bioinformatics analysis

Data of *SETD1A* DNA copy number in lung cancer is from cBioportal ([www.cbiportal.org](http://www.cbiportal.org)). Gene expression data from GEO DataSets ([https://www.ncbi.nlm.nih.gov/gds/](https://www.ncbi.nlm.nih.gov/gds/)) and the accession number is listed in figure legends. Data of cumulative overall survival of patients with lung carcinoma is from TCGA database. *SETD1A* expression in different cell line is from previous report\textsuperscript{12}. Enhanced H3K4me3 signaling in cell line of lung cancer was confirmed with ENCODE ([https://www.encodeproject.org/](https://www.encodeproject.org/)), the database collects and verifies CHIP-seq results.

Statistical analysis

Data are presented as the mean ± SD, except for description in figure legends. Number of biological repetition are also stated in figure legends. The difference between unpaired groups was confirmed with two-tailed Welch's t test. Log-rank (Mantel-Cox) test was performed in analysis of survival curves. For comparison of cell number and ratio, log transformation was used for statistical test. The confidence level was 95%. All statistical analysis was performed by GraphPad Prime 7.00 software.

Abbreviations

H3K4me3\textsuperscript{\textendash}methylation of histone 3 lysine 4

*SETD1A*\textsuperscript{\textendash}SET domain containing 1A

EMT\textsuperscript{\textendash}epithelial-mesenchymal transition

Declarations

Ethics approval and consent to participate

The animal experiments were approved by the medical ethics committee of Qilu hospital of XXXX University.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no potential conflicts of interest.
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Authors' contributions

M.D. and X.W.W. conceived of the study, designed the study and collected the data. All authors analysed the data and were involved in writing the manuscript.

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Conflict of interest

The authors declare no potential conflicts of interest.

Ethics

The animal experiments were approved by the medical ethics committee of Qilu hospital of Shandong University.

References

1. Siegel, R. L., Miller, K. D., and Jemal, A. (2019) Cancer statistics, 2019. CA Cancer J. Clin. 69, 7-34
2. Global Burden of Disease Cancer, C., Fitzmaurice, C., Allen, C., Barber, R. M., Barregard, L., Bhatta, Z. A., Brenner, H., Dicker, D. J., Chimed-Orchir, O., Dandona, R., Dandona, L., Fleming, T., Forouzanfar, M. H., Hancock, J., Hay, R. J., Hunter-Merrill, R., Huynh, C., Hosgood, H. D., Johnson, C. O., Jonas, J. B., Khubchandani, J., Kumar, G. A., Kutz, M., Lan, Q., Larson, H. J., Liang, X., Lim, S. S., Lopez, A. D., MacIntyre, M. F., Marczak, L., Marquez, N., Mokdad, A. H., Pinho, C., Pourmalek, F., Salomon, J. A., Sanabria, J. R., Sandar, L., Sartorius, B., Schwartz, S. M., Shackelford, K. A., Shibuya, K., Stanaway, J., Steiner, C., Sun, J., Takahashi, K., Vollset, S. E., Vos, T., Wagner, J. A., Wang, H., Westerman, R., Zeeb, H., Zoeckler, L., Abd-Allah, F., Ahmed, M. B., Alabeled, S., Alam, N. K., Aldhahri, S. F., Alem, G., Alemayohu, M. A., Ali, R., Al-Raddadi, R., Amare, A., Amoako, Y., Artaman, A., Asayesh, H., Atanafu, N., Awasthi, A., Saleem, H. B., Barac, A., Bedi, N., Bensenor, I., Berhane, A., Bernabe, E., Betsu, B., Binagwaho, A., Boney, D., Campos-Nonato, I., Castaneda-Orjuela, C., Catala-Lopez, F., Chiang, P., Chibueze, C., Chitheer, A., Choi, J. Y., Cowie, B., Damtew, S., das Neves, J., Dey, S., Dharmaratne, S., Dhillon, P., Ding, E., Driscoll, T., Ekwueme, D., Endries, A. Y., Farvid, M., Farzadfar, F., Fernandes, J.,
Fischer, F., TT, G. H., Gebru, A., Gopalani, S., Hailu, A., Horino, M., Horita, N., Husseini, A., Huybrechts, I., Inoue, M., Islami, F., Jakovljevic, M., James, S., Javanbakht, M., Jee, S. H., Kasaeian, A., Kedir, M. S., Khader, Y. S., Khang, Y. H., Kim, D., Leigh, J., Linn, S., Lunevicius, R., El Razek, H. M. A., Malekzadeh, R., Malta, D. C., Marcenes, W., Markos, D., Melaku, Y. A., Meles, K. G., Mendoza, W., Mengiste, D. T., Meretoja, T. J., Miller, T. R., Mohammad, K. A., Mohammad, S., Moradi-Lakeh, M., Nagel, G., Nand, D., Le Nguyen, Q., Nolte, S., Ogbo, F. A., Oladimeji, K. E., Oren, E., Pa, M., Park, E. K., Pereira, D. M., Plass, D., Qorbani, M., Radfar, A., Rafay, A., Rahman, M., Rana, S. M., Soreide, K., Satpathy, M., Sawhney, M., Sepanlou, S. G., Shaikh, M. A., She, J., Shiue, I., Shore, H. R., Shrive, M. G., So, S., Soneji, S., Stathopoulos, V., Stroumpoulis, K., Sufiyan, M. B., Sykes, B. L., Tabares-Seisdedos, R., Tadesse, F., Tedla, B. A., Tessema, G. A., Thakur, J. S., Tran, B. X., Ukwaja, K. N., Uzochukwu, B. S. C., Vlassov, V. V., Weiderpass, E., Wubshet Terefe, M., Yebyo, H. G., Yimam, H. H., Yonemoto, N., Younis, M. Z., Yu, C., Zaidi, Z., Zaki, M. E. S., Zenebe, Z. M., Murray, C. J. L., and Naghavi, M. (2017) Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA oncology* 3, 524-548

3. Herbst, R. S., Morgensztern, D., and Boshoff, C. (2018) The biology and management of non-small cell lung cancer. *Nature* 553, 446-454

4. Mayekar, M. K., and Bivona, T. G. (2017) Current Landscape of Targeted Therapy in Lung Cancer. *Clin. Pharmacol. Ther.* 102, 757-764

5. Jamal-Hanjani, M., Wilson, G. A., McGranahan, N., Birkbak, N. J., Watkins, T. B. K., Veeriah, S., Shafi, S., Johnson, D. H., Mitter, R., Rosenthal, R., Salm, M., Horswell, S., Escudero, M., Matthews, N., Rowan, A., Chambers, T., Moore, D. A., Turajlic, S., Xu, H., Lee, S. M., Forster, M. D., Ahmad, T., Hiley, C. T., Abbosh, C., Falzon, M., Borg, E., Marafioti, T., Lawrence, D., Hayward, M., Kolvekar, S., Panagiotopoulos, N., Janes, S. M., Thakrar, R., Ahmed, A., Blackhall, F., Summers, Y., Shah, R., Joseph, L., Quinn, A. M., Crosbie, P. A., Naidu, B., Middleton, G., Langman, G., Trotter, S., Nicolson, M., Remmen, H., Kerr, K., Chetty, M., Gomersall, L., Fennell, D. A., Nakas, A., Rathinam, S., Anand, G., Khan, S., Russell, P., Ezhil, V., Ismail, B., Irvin-Sellers, M., Prakash, V., Lester, J. F., Kornaszewska, M., Attanoos, R., Adams, H., Davies, H., Dentro, S., Taniere, P., O'Sullivan, B., Lowe, H. L., Hartley, J. A., Illes, N., Bell, H., Ngai, Y., Shaw, J. A., Herrera, J., Szallasi, Z., Schwarz, R. F., Stewart, A., Quezada, S. A., Le Quesne, J., Van Loo, P., Dive, C., Hackshaw, A., Swanton, C., and Consortium, T. R. (2017) Tracking the Evolution of Non-Small-Cell Lung Cancer. *N. Engl. J. Med.* 376, 2109-2121

6. Widschwendter, M., Jones, A., Evans, I., Reisel, D., Dillner, J., Sundstrom, K., Steyerberg, E. W., Vergouwe, Y., Wegwarth, O., Rebitschek, F. G., Siebert, U., Sroczyński, G., de Beaumont, I. D., Bolt, I., Cibula, D., Zikan, M., Bjorge, L., Colombo, N., Harbeck, N., Dudbridge, F., Tasse, A. M., Knoppers, B. M., Joly, Y., Teschendorff, A. E., Pashayan, N., and Consortium, F. (2018) Epigenome-based cancer risk prediction: rationale, opportunities and challenges. *Nat. Rev. Clin. Oncol.* 15, 292-309

7. Smith, E., and Shilatifard, A. (2010) The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. *Molecular cell* 40, 689-701
8. Takeshima, H., Wakabayashi, M., Hattori, N., Yamashita, S., and Ushijima, T. (2015) Identification of coexistence of DNA methylation and H3K27me3 specifically in cancer cells as a promising target for epigenetic therapy. *Carcinogenesis* **36**, 192-201

9. Kuntimaddi, A., Achille, N. J., Thorpe, J., Lokken, A. A., Singh, R., Hemenway, C. S., Adli, M., Zeleznik-Le, N. J., and Bushweller, J. H. (2015) Degree of recruitment of DOT1L to MLL-AF9 defines level of H3K79 Di- and tri-methylation on target genes and transformation potential. *Cell reports* **11**, 808-820

10. Gao, Y., Zhao, Y., Zhang, J., Lu, Y., Liu, X., Geng, P., Huang, B., Zhang, Y., and Lu, J. (2016) The dual function of PRMT1 in modulating epithelial-mesenchymal transition and cellular senescence in breast cancer cells through regulation of ZEB1. *Sci. Rep.* **6**, 19874

11. Li, S., Shen, L., and Chen, K. N. (2018) Association between H3K4 methylation and cancer prognosis: A meta-analysis. *Thoracic cancer* **9**, 794-799

12. Kikutake, C., and Yahara, K. (2016) Identification of Epigenetic Biomarkers of Lung Adenocarcinoma through Multi-Omics Data Analysis. *PLoS One* **11**, e0152918

13. Suzuki, A., Makinoshima, H., Wakaguri, H., Esumi, H., Sugano, S., Kohno, T., Tsuchihara, K., and Suzuki, Y. (2014) Aberrant transcriptional regulations in cancers: genome, transcriptome and epigenome analysis of lung adenocarcinoma cell lines. *Nucleic Acids Res* **42**, 13557-13572

14. Shilatifard, A. (2012) The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. *Annual review of biochemistry* **81**, 65-95

15. Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A., and Shi, Y. (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119**, 941-953

16. Secombe, J., Li, L., Carlos, L., and Eisenman, R. N. (2007) The Trithorax group protein Lid is a trimethyl histone H3K4 demethylase required for dMyc-induced cell growth. *Genes Dev.* **21**, 537-551

17. Tahiliani, M., Mei, P., Fang, R., Leonor, T., Rutenberg, M., Shimizu, F., Li, J., Rao, A., and Shi, Y. (2007) The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation. *Nature* **447**, 601-605

18. Yamane, K., Tateishi, K., Klose, R. J., Fang, J., Fabrizio, L. A., Erdjument-Bromage, H., Taylor-Papadimitriou, J., Tempst, P., and Zhang, Y. (2007) PLU-1 is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. *Mol. Cell* **25**, 801-812

19. Lee, M. G., Norman, J., Shilatifard, A., and Shiekhattar, R. (2007) Physical and functional association of a trimethyl H3K4 demethylase and Ring6a/MBLR, a polycomb-like protein. *Cell* **128**, 877-887

20. Morera, L., Lubbert, M., and Jung, M. (2016) Targeting histone methyltransferases and demethylases in clinical trials for cancer therapy. *Clin. Epigenetics* **8**, 57

21. Salz, T., Deng, C., Pampo, C., Siemann, D., Qiu, Y., Brown, K., and Huang, S. (2015) Histone Methyltransferase hSETD1A Is a Novel Regulator of Metastasis in Breast Cancer. *Molecular cancer research: MCR* **13**, 461-469

22. Salz, T., Li, G., Kaye, F., Zhou, L., Qiu, Y., and Huang, S. (2014) hSETD1A regulates Wnt target genes and controls tumor growth of colorectal cancer cells. *Cancer research* **74**, 775-786
23. Selamat, S. A., Chung, B. S., Girard, L., Zhang, W., Zhang, Y., Campan, M., Siegmund, K. D., Koss, M. N., Hagen, J. A., Lam, W. L., Lam, S., Gazdar, A. F., and Laird-Offringa, I. A. (2012) Genome-scale analysis of DNA methylation in lung adenocarcinoma and integration with mRNA expression. *Genome Res.* **22**, 1197-1211

24. Rapp, J., Jaromi, L., Kvell, K., Miskei, G., and Pongracz, J. E. (2017) WNT signaling - lung cancer is no exception. *Respir. Res.* **18**, 167

25. Jiang, R., Jin, Z., Liu, Z., Sun, L., Wang, L., and Li, K. (2011) Correlation of activated STAT3 expression with clinicopathologic features in lung adenocarcinoma and squamous cell carcinoma. *Mol. Diagn. Ther.* **15**, 347-352

26. Linnerth-Petrik, N. M., Santry, L. A., Petrik, J. J., and Wootton, S. K. (2014) Opposing functions of Akt isoforms in lung tumor initiation and progression. *PLoS One* **9**, e94595

27. Eser, P. O., and Janne, P. A. (2018) TGFbeta pathway inhibition in the treatment of non-small cell lung cancer. *Pharmacology & therapeutics* **184**, 112-130

28. Zaidi, S. K., Frietze, S. E., Gordon, J. A., Heath, J. L., Messier, T., Hong, D., Boyd, J. R., Kang, M., Imbalzano, A. N., Lian, J. B., Stein, J. L., and Stein, G. S. (2017) Bivalent Epigenetic Control of Oncofetal Gene Expression in Cancer. *Mol. Cell. Biol.* **37**

29. Zhang, J., Zhang, J., Cui, X., Yang, Y., Li, M., Qu, J., Li, J., and Wang, J. (2015) FoxM1: a novel tumor biomarker of lung cancer. *Int. J. Clin. Exp. Med.* **8**, 3136-3140

30. Bragelmann, J., Bohm, S., Guthrie, M. R., Mollaoglu, G., Oliver, T. G., and Sos, M. L. (2017) Family matters: How MYC family oncogenes impact small cell lung cancer. *Cell Cycle* **16**, 1489-1498

31. Cao, L. L., Song, X., Pei, L., Liu, L., Wang, H., and Jia, M. (2017) Histone deacetylase HDAC1 expression correlates with the progression and prognosis of lung cancer: A meta-analysis. *Medicine (Baltimore)* **96**, e7663

32. Lai, Q., Xu, Y. H., Chen, Q., Tang, L., Li, A. G., Zhang, L. F., Zhang, C. F., Song, J. F., and Du, Z. Z. (2017) The loss-of-function of DNA methyltransferase 1 by siRNA impairs the growth of non-small cell lung cancer with alleviated side effects via reactivation of RASSF1A and APC in vitro and vivo. *Oncotarget* **8**, 59301-59311

33. Sun, Q. Y., Ding, L. W., Xiao, J. F., Chien, W., Lim, S. L., Hattori, N., Goodglick, L., Chia, D., Mah, V., Alavi, M., Kim, S. R., Doan, N. B., Said, J. W., Loh, X. Y., Xu, L., Liu, L. Z., Yang, H., Hayano, T., Shi, S., Xie, D., Lin, D. C., and Koeffler, H. P. (2015) SETDB1 accelerates tumourigenesis by regulating the WNT signalling pathway. *J Pathol* **235**, 559-570

34. Mastrangelo, E., and Milani, M. (2018) Role and inhibition of GLI1 protein in cancer. *Lung Cancer (Auckl)* **9**, 35-43