***Cell Lines and Reagents***

Culture information of 55 NSCLC, SCLC, HREC cell lines used in this paper were summarized in Supplementary Table S1 in Supplementary Digital Content 2. Cell line provenance was confirmed by DNA genotyping using the StemElite ID system (Promega Corporation, Madison, WI) and by comparison with the reference genotypes in our database.

Antibodies against HA (Covance, Princeton, NJ), ITPKA (Proteintech, Chicago, IL), DNMT1 and DNMT3A (Cell Signaling, Danvers, MA), DNMT3B (Abcam, Cambridge, UK) and GAPDH (GeneTex, Irvine, CA) were used in western blot analyses. SP1 antibody (Cell Signaling) was used for chromatin-immunoprecipitation. 5-aza-2'-deoxycytidine (5-aza-dC) was purchased from Tocris, Minneapolis, MN.

***Constructs and Cell Transfection***

ITPKA expression vector was constructed by PCR amplification of the full-length cDNA of *ITPKA* using Fetchmygene (Transomic, Huntsville, AL) as the template, followed by cloning the PCR product into pGM-T vector and then into pcDNA3.0-HA vector (Life technologies, NY). For ITPKA overexpression, NCI-H2009 and NCI-H1299 lung cancer cell lines were individually transfected with pcDNA3.0-HA-ITPKA or HA plasmid alone by LF2000 (Life Technologies). Cell

clones stably expressing ectopic HA-ITPKA or harboring the HA plasmid were established by culturing the transfected cells in medium in the presence of G418 (Sigma) for 14 days.

For reporter assays, the pGL3-*ITPKA* promoter (p*ITPKA*pro-Luc) plasmid was constructed by PCR amplification of *ITPKA* promoter region (chr15: 41784556-41786055 bps) from BAC clone RP11-1609 (GenDiscovery Biotechnology, Taipei, Taiwan) using gene-specific primers (Supplementary Materials & Methods Table S3), followed by inserting the PCR product into pGM-T vector, and then into pGL3 vector (Promega, Madison, WI). The p*ITPKA*pro-Luc-Me plasmid, which contains an additional *ITPKA* body methylation region in the reporter plasmid, was constructed by PCR amplification of the *ITPKA* CpG island-2 region using BAC clone RP11-1609 as template, followed by inserting the PCR product into p*ITPKA*pro-Luc at position 270 bps downstream the luciferase coding region at SalI restriction site.

***Gene Knockdown Experiment***

For knock down experiments, cells were transiently transfected with *ITPKA* siRNA oligomer (Life Technologies) or a control siRNA oligomer using Lipofectamine RNAiMAX reagent (Life Technologies). Seventy-two hours post

transfection, cells were harvested for western blot and oncogenic transformation analyses. Alternatively, lentivirus-based knock down approach was performed to eliminate the expression of designated genes in various cell lines. Lentiviral pLKO.1 vectors harboring individual shRNA against *ITPKA* (target sequence: CTTTCCACCTCGTCGGTCTC), *DNMT1* (target sequence: GAGGTTCGCTTATCAACTAAT), *DNMT3A* (target sequence: CCCAAGGTCAAGGAGATTATT) and *DNMT3B* (target sequence: GCAGGCAGTAGGAAATTAGAA) were purchased from National RNAi Core Facility, Taiwan. Briefly, pLKO.1-shRNA plasmid was cotransfected with packing plasmids pMD.G and pCMV $\Delta$ R8.91 into 293T cells by LF2000 to produce virus-containing medium. Viruses were collected from the medium 60 h after transfection. For knock down experiments, cells were infected with the collected viruses over 24 h in the presence of polybrene, followed by selection in medium containing puromycin (5 μg/mL) for 14 days.

***Quantitative PCR***

Total RNA was prepared from homogenized tissues or cells and converted to cDNA as described previously.[1] *ITPKA* expression was measured by quantitative polymerase chain reaction (qPCR) using Taqman Gene Expression Master Mix (Life

Technologies, Waltham) with *ITPKA* specific primers (Life Technologies), and presented in a ratio in relation to the expression of TATA-binding protein as *ITPKA*/*TBP* ratio × 1000 as described previously.[2]

***Cell Growth and Colony Formation Assays***

Cell growth assay was performed by seeding $1\times10^3$ cells in each well in 96-well plate, with fresh medium replenished every other day. To measure the cell number at designated time, 10μl of Cell Counting Kit-8 reagent (Sigma) was added to each well, and the absorbance at 450nm was measured after incubation for 70 min. For colony formation assay, cells were seeded in 6-well plate (150 cells per well), with medium replaced with fresh medium every two days. After 12 days, cells were stained with crystal violet (0.3% w/v in 20% ethanol). The number of colonies, defined as >50 cells/colony, was counted by Image J software.

***Cell Migration and Invasion Assays***

Cell migration and invasion was analyzed by Boyden chamber assay as described previously.[3]

***Luciferase Reporter Assay***

*ITPKA* promoter activity was measured by using a dual-luciferase assay kit (Promega, Madison). Briefly, cells were co-transfected with the indicated reporter plasmid along with pRL-TK. After transfection, cells were harvested and luciferase activity was measured.

***In Vivo Xenograft Tumor Formation Assay***

All experimental procedures were carried out in accordance with approved guidelines of the Institutional Animal Care and Utilization Committee at Academia Sinica, Taiwan. A total of $1 \times 10^6$ cells were subcutaneously injected into the right flank of 5-week-old NOD-*scid IL2rγ*$^{null}$ (NSG) mice. Tumor size was measured weekly and tumor volume was calculated according to the formula: volume = length × width$^2$ × 0.52.

***Human Samples***

Clinical information of patients was organized in Supplementary Table S2 of Supplementary Digital Content 2. All samples were from patients undergone surgical resections for malignant or other pathologic conditions. Gene expression data (HumanHT-12 v3 Expression BeadChip-Illumina) were obtained from 83 pairs of lung adenocarcinomas and their corresponding non-malignant lung tissues by Dr.

Stephen Lam, University of British Columbia, Vancouver, Canada.[4] Other surgically resected lung tissues were obtained from M. D. Anderson Cancer Center. Peripheral blood cells were obtained from healthy adults to serve as negative controls in bi-sulfite sequencing analysis at UT Southwestern Medical Center. For determining the role of *ITPKA* methylation during the multistage pathogenesis of lung adenocarcinomas, formalin-fixed paraffin embedded (FFPE) samples were obtained from the Aiichi Cancer Center, Nagoya, Japan, and two pathologists (YY and AFG) independently verified the histological diagnoses. Thirteen non-malignant lung samples (including histologically normal, COPD and chronic inflammatory lesions) were also included. For determining the role of *ITPKA* methylation during the multistage pathogenesis of lung squamous cell carcinoma, 4 paired FFPE archives (2 pairs of hyperplasia (HYP) and dysplasia (DYS), 2 pairs of hyperplasia and carcinoma in situ (CIS) were obtained from the Vancouver General Hospital, Vancouver, Canada and independently verified with the histological diagnosis by two experienced pathologists (AFG and local pathologist). The squamous FFPE archives were collected from volunteers of heavy smokers above 45 years of age with 20 ≥ packs per year smoking history without previously detected cancer in the upper aerodigestive tract when they were screened by autofluorescence bronchoscopy prior to enrollment into a chemoprevention trial.[5]

***Genomic DNA Extraction and Methylation Level Measurement***

Genomic DNA was prepared by using QIAamp DNA Blood Mini Kit (Qiagen, Hilden). Alternatively, the DNA from formalin-fixed, paraffin-embedded (FFPE) "curls" was isolated using ALLPrep DNA/RNA FFPE kit (Qiagen). The bisulfite modification of genomic DNA was performed using EZ DNA Methylation-Gold kit (Zymo Research, Irvine) as described previously.[6, 7]

DNA methylation was analyzed by bi-sulfite sequencing, quantitative methylation-specific polymerase chain reaction (qMSP) and 450K methylation array. For bisulfite sequencing analysis, bisulfite-modified DNA was amplified by PCR reaction and subjected to sequencing in both directions (Supplementary Table S3 in Supplementary Digital Content 2). To quantitate the level of methylation at individual CpG sites, the peak heights of the corresponding cytosine (C) and thymine (T) signals of each CpG site were measured, and the relative incidence of cytosine calculated as: C (%) = Peak height of C/(Peak height of C + Peak height of T) × 100 as previously described, [8] and Finch TV software (PerkinElmer, Seattle, WA) was used. Alternatively, methylation levels of *ITPKA* gene body were quantified by qMSP. The bisulfite-modified DNA was amplified using Taqman Gene expression master mix in the presence of *ITPKA*-specific qMSP primers and probe (Supplementary Table S3 in

Supplementary Digital Content 2). *β-actin* and *RNase P* served as reference genes for cell lines & frozen primary samples, and for FFPE, respectively. The *ITPKA* body methylation was quantitatively calculated by the method as described previously,[7] as a methylation ratio (*ITPKA*/*β-actin* × 1000 or *ITPKA*/*RNase P* × 1000). The Infinium HumanMethylation 450 BeadChip (450K methylation array) was also performed to assess the genome-wide DNA methylation according to the manufacturer's procedures. The β-values were the estimate of methylation levels using ratio of intensities between methylated and unmethylated alleles. The CpG site was considered heavily methylated when β-values ≥ 0.6, and partially methylated when 0.6 > β-values > 0.2, and unmethylated when β-values ≤ 0.2.

***Statistical Analyses***

Details of the statistical analyses used in this paper were described in Supplemental Digital Content 1. The Mann-Whitney *U* test was performed to test the difference in the quantitation of the levels of methylation and gene expression. The differences of *in vitro* cell proliferation rate and *in vivo* xenograft tumor formation were analyzed by two-way Anova. Student's *t* test was used to determine the differences between the control and test groups (*, when $p< 0.05$; **, when $p< 0.01$; and ***, when $p< 0.001$). Spearman correlation coefficient (*r*) was calculated to

measure the correlation. Receptor operating characteristics (ROC) curves were used to indicate the accuracy of a marker in distinguishing cancers from non-malignant in tissues and cell lines, and the area under the curve (AUC) was also included. The Cochran-Armitage Test for Trend was used for determining progressive *ITPKA* methylation during multistage pathogenesis of lung carcinoma.

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# Supplementary Table

## Supplementary Table S1. Top 20 upregulated genes in 83 pairs of lung adenocarcinomas and non-tumorous samples

| Rank | Gene name | Symbol | Accession | Fold activation |
|---|---|---|---|---|
| 1 | X antigen family, member 1D | XAGE1D | NM_133431 | 23.46 |
| 2 | Matrix metallopeptidase 11 | MMP11 | NM_005940 | 21.25 |
| 3 | Eukaryotic translation elongation factor 1 alpha 2 | EEF1A2 | NM_001958 | 21.04 |
| 4 | Glucosaminyl (N-acetyl) transferase 3, mucin type | GCNT3 | NM_004751 | 19.73 |
| 5 | Cystatin SN | CST1 | NM_001898 | 17.94 |
| 6 | Family with sequence similarity 83, member A | FAM83A | NM_032899 | 16.57 |
| 7 | Collagen, type XI, alpha 1 | COL11A1 | NM_080629 | 15.12 |
| 8 | Serine peptidase inhibitor, Kazal type 1 | SPINK1 | NM_003122 | 15.10 |
| 9 | Secreted phosphoprotein 1 | SPP1 | NM_000582 | 14.87 |
| 10 | Inositol 1,4,5-trisphosphate 3-kinase A | ITPKA | NM_002220 | 13.45 |
| 11 | Topoisomerase (DNA) II alpha | TOP2A | NM_001067 | 11.43 |
| 12 | Ets variant 4 | ETV4 | NM_001986 | 10.93 |
| 13 | Cellular retinoic acid binding protein 2 | CRABP2 | NM_001878 | 10.81 |
| 14 | Paired-like homeodomain 1 | PITX1 | NM_002653 | 10.52 |
| 15 | Cartilage oligomeric matrix protein | COMP | NM_000095 | 9.92 |
| 16 | Ubiquitin-conjugating enzyme E2C | UBE2C | NM_181800 | 9.82 |
| 17 | Transcription factor AP-2 alpha | TFAP2A | NM_001032280 | 8.58 |
| 18 | Pyrroline-5-carboxylate reductase 1 | PYCR1 | NM_153824 | 8.45 |
| 19 | Tubulin, beta 3 | TUBB3 | NM_006086 | 8.38 |
| 20 | Carcinoembryonic antigen-related cell adhesion molecule 5 | CEACAM5 | NM_004363 | 8.29 |

Top 20 upregulated genes are listed from gene expression data of cDNA microarray (HumanHT-12 v3 Expression BeadChip-Illumina) from 83 pairs of resected lung adenocarcinomas and corresponding non-malignant lung tissues.

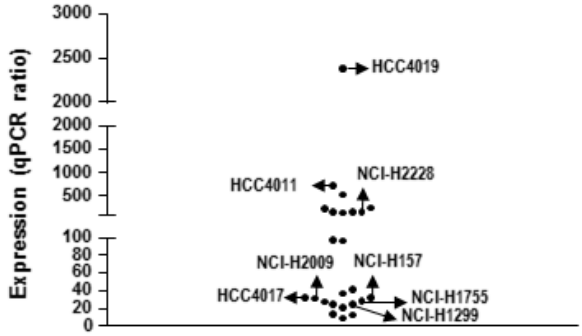
## Supplementary Table S2. β-value of *ITPKA* probes of 450K methylation array in cell lines and lung cancers

| Description | Probe name | Probe position | Methylation (β-value) | | | | p-value | |
|---|---|---|---|---|---|---|---|---|
| | | | HBEC & HSAEC (n=28) | Lung cancer cell lines (n=158) | TCGA | | HBEC & HSAEC vs. Lung cancer cell lines | TCGA |
| | | | | | Non-malignant (n=75) | ADC + SCC (n=825) | | Non-malignant vs. ADC+ SCC |
| CpG island-1 | cg25609143 | TSS1500 | 0.04 | 0.04 | 0.02 | 0.02 | 0.3638 | 5.4254E-07 |
| | cg25933094 | TSS200 | 0.05 | 0.05 | NA | NA | 0.1229 | NA |
| | cg02330494 | 1stExon | 0.05 | 0.06 | 0.02 | 0.03 | 0.0556 | 1.42E-03 |
| | cg01808706 | Body | 0.19 | 0.18 | NA | NA | 0.1571 | NA |
| | cg07381778 | Body | 0.03 | 0.07 | 0.02 | 0.02 | 0.0002 | 3.50E-02 |
| | cg12386646 | Body | 0.85 | 0.81 | 0.17 | 0.29 | 0.0655 | 2.72E-13 |
| | cg20272979 | Body | 0.88 | 0.89 | 0.12 | 0.48 | 0.2982 | 8.58E-22 |
| | cg02330683 | Body | 0.81 | 0.84 | 0.44 | 0.65 | 0.0007 | 1.26E-15 |
| CpG island-2 | cg09299055 | Body | 0.49 | 0.91 | 0.13 | 0.46 | 8.80084E-15 | 5.37203E-41 |
| | cg08680048 | Body | 0.26 | 0.97 | 0.03 | 0.47 | 3.17452E-16 | 1.00958E-44 |
| | cg03927133 | Body | 0.42 | 0.86 | 0.22 | 0.42 | 2.01861E-21 | 6.41744E-37 |
| | cg11789612 | Body | 0.42 | 0.97 | 0.11 | 0.54 | 5.38337E-22 | 2.54248E-73 |
| | cg03177551 | Body | 0.17 | 0.82 | 0.13 | 0.37 | 1.39707E-24 | 8.86955E-28 |
| | cg27501645 | Body | 0.37 | 0.97 | 0.42 | 0.52 | 9.78694E-30 | 5.82E-15 |

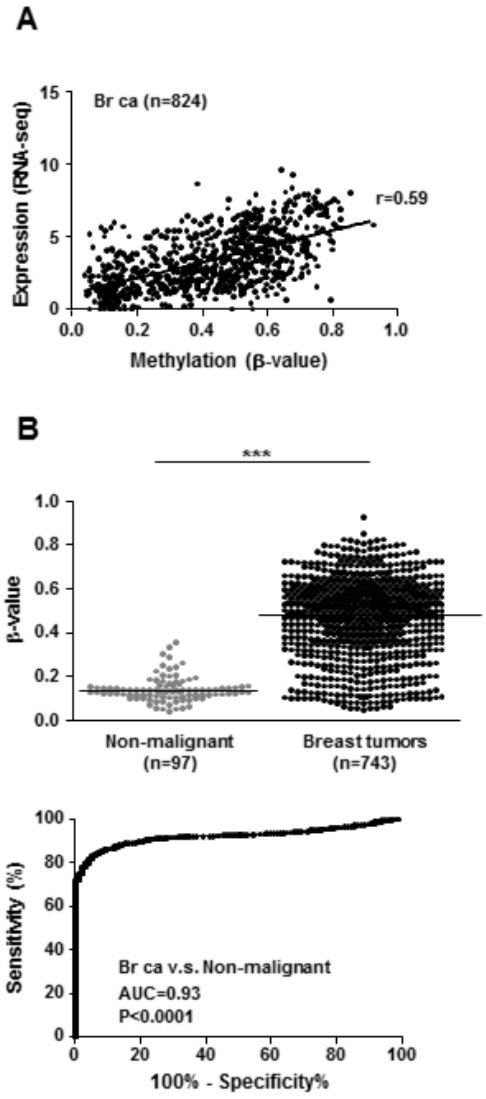
Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma; TSS: transcription start site; NA: not available

NA: not avaliable

## Supplementary Figures

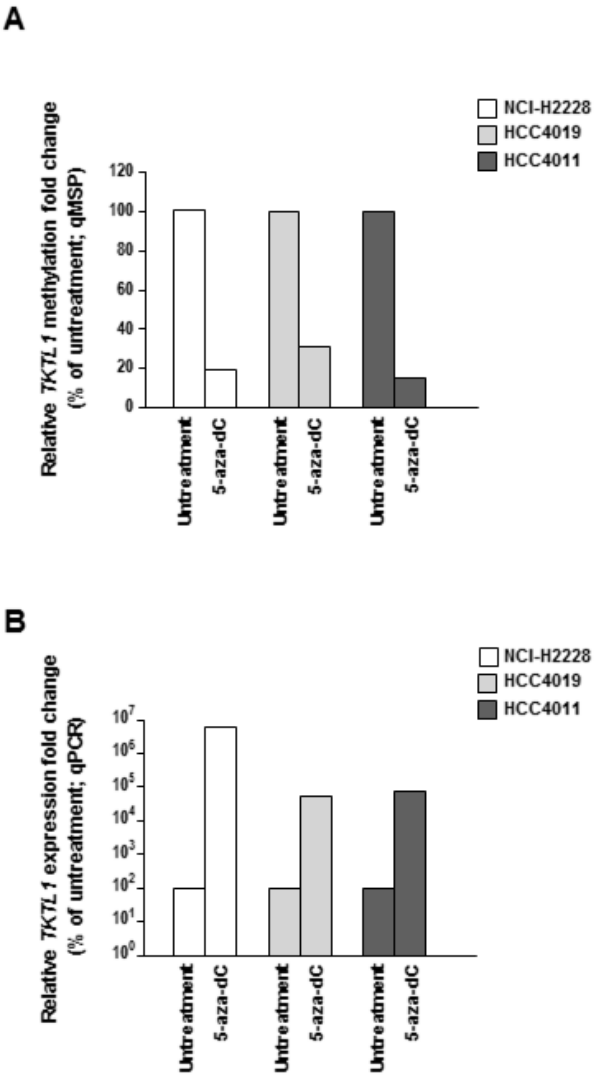



**Supplementary Fig S1. *ITPKA* gene expression and the levels of gene body methylation in NSCLC cell lines.** *ITPKA* expression by qPCR analysis of 23 NSCLC cell lines.

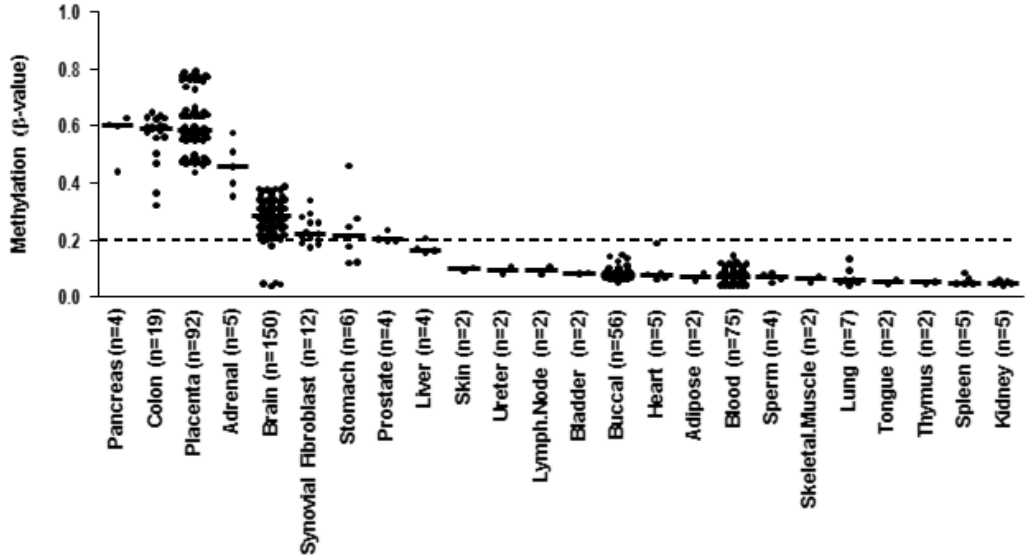



**Supplementary Fig S2. *ITPKA* body methylation is positively correlated with gene expression, and may serve as a diagnostic biomarker for breast cancer detection. A,** *ITPKA* expression was determined by RNA-seq and methylation was measured by 450K methylation array in 462 ADC and 365 SCC. Methylation levels were assessed as the average of the β-values of the five probes cg09299055, cg08680048, cg03927133, cg11789612 and cg03177551. Pearson's correlation coefficient was included. **B,** methylation levels of *ITPKA* gene body detected by 450K

methylation array in primary breast cancers. The Receptor Operating Characteristic (ROC) curve analysis is shown, and the AUC score and p-value are also included.




**Supplementary Fig. S3. Effects of 5-aza-dC on *TKTL1* expression. A-B,** Effects of 5-aza-dC treatment on the promoter methylation (A) and expression (B) of *TKTL1* in high *ITPKA*-expressing cells. Cells were cultured in the presence 5-aza-dC for five days. Gene body methylation determined by qMSP analysis and *ITPKA* expression by qPCR method are shown in relation to those derived from the untreated samples. Data are shown as mean ± SD.




**Supplementary Fig. S4. *ITPKA* gene body methylation in normal tissues by 450K methylation array.** Methylation levels of 469 normal samples derived from 24 different tissues were determined using the average of the β-values of five probes (cg09299055, cg08680048, cg03927133, cg11789612 and cg03177551) in the 450K methylation array. The median values are indicated by black vertical lines.

**Supplementary Materials & Methods Table S2. Cultured information of cell lines**

| Cell type | Cell | Maintained medium |
|---|---|---|
| NSCLC | NCI-H1299 | RPMI+10% FBS+ antibiotic |
| | NCI-H2009 | |
| | NCI-H2228 | |
| | NCI-HCC4019 | |
| | NCI-HCC4011 | |
| | NCI-HCC4017 | |
| | NCI-H157 | |
| | NCI-H1755 | |
| | NCI-H2073 | |
| | NCI-H322 | |
| | NCI-H1993 | |
| | NCI-H1792 | |
| | NCI-H1974 | |
| | NCI-H1373 | |
| | NCI-H441 | |
| | NCI-H125 | |
| | NCI-H1570 | |
| | NCI-HCC15 | |
| | NCI-H1573 | |
| | NCI-H2122 | |
| | NCI-A549 | |
| | NCI-HCC4054 | |
| | NCI-HCC44 | |

| Cell type | Cell | Maintained medium |
|---|---|---|
| SCLC | NCI-H1876 | RPMI+10% FBS+ antibiotic |
| | NCI-H69 | |
| | NCI-HCC4000 | |
| | NCI-HCC4001 | |
| | NCI-H524 | |
| | NCI-H1694 | |
| | NCI-HCC41 | |
| | NCI-H1436 | |
| | NCI-H289 | |
| | NCI-H2171 | |
| | NCI-H526 | |
| | NCI-H1963 | |
| | NCI-HCC970 | |
| | NCI-HCC4003 | |
| | NCI-HCC1819 | |
| | NCI-HCC4002 | |
| | NCI-HCC2433 | |
| | NCI-HCC4005 | |
| | NCI-HCC33 | |
| | NCI-H1238 | |

| Cell type | Cell | Maintained medium |
|---|---|---|
| HRECs | HBEC17-KT | Keratinocyte-SFM (Gibco; #10724) |
| | HBEC13-KT | |
| | HBEC34-KT | |
| | HBEC-C30-KT | |
| | HBEC-C3-KT | |
| | HBEC-C2-KT | |
| | HSAEC97-KT | Small airway cell basal medium (Lonza; #CC-3119) |
| | HSAEC31-KT | |
| | HSAEC37-KT | |
| | HSAEC1-KT | |
| | HSAEC19-KT | |
| | HSAEC18-KT | |

NSCLC: non-small cell lung cancer, SCLC: small cell lung cancer, HRECs: immortalized human respiratory epithelial cells

**Supplementary Materials & Methods Table S1. Patient clinical information**

| Sample | Experiment | Resource | Case ID (IW) | Tissue (Tumor or Normal) | Diagnosis | Sample ID (IW) |
|---|---|---|---|---|---|---|
| Genomic DNA(MDAC) | qMSP | MDAC | 2305 | N | Adenocarcinoma | MDA-2305-N |
| | | | 2081 | N | Adenocarcinoma | MDA-2081-N |
| | | | 1487 | N | Adenocarcinoma | MDA-1487-N |
| | | | 1936 | N | Adenocarcinoma | MDA-1936-N |
| | | | 2271 | N | Adenocarcinoma | MDA-2271-N |
| | | | 2317 | N | Adenocarcinoma | MDA-2317-N |
| | | | 2249 | N | Adenocarcinoma | MDA-2249-N |
| | | | 2072 | N | Adenocarcinoma | MDA-2072-N |
| | | | 2309 | N | Adenocarcinoma | MDA-2309-N |
| | | | 1942 | N | Adenocarcinoma | MDA-1942-N |
| | | | 1193 | N | Adenocarcinoma | MDA-1193-N |
| | | | 2291 | N | Adenocarcinoma | MDA-2291-N |
| | | | 1654 | N | Adenocarcinoma | MDA-1654-N |
| | | | 1998 | N | Adenocarcinoma | MDA-1998-N |
| | | | 1989 | N | Adenocarcinoma | MDA-1989-N |
| | | | 2332 | N | Adenocarcinoma | MDA-2332-N |
| | | | 2230 | T | Adenocarcinoma | MDA-2230-T |
| | | | 2081 | T | Adenocarcinoma | MDA-2081-T |
| | | | 2102 | T | Adenocarcinoma | MDA-2102-T |
| | | | 1240 | T | Adenocarcinoma | MDA-1240-T |
| | | | 1186 | T | Adenocarcinoma | MDA-1186-T |
| | | | 2309 | T | Adenocarcinoma | MDA-2309-T |
| | | | 2159 | T | Adenocarcinoma | MDA-2159-T |
| | | | 2271 | T | Adenocarcinoma | MDA-2271-T |
| | | | 1747 | T | Adenocarcinoma | MDA-1747-T |
| | | | 1193 | T | Adenocarcinoma | MDA-1193-T |
| | | | 1522 | T | Adenocarcinoma | MDA-1522-T |
| | | | 1936 | T | Adenocarcinoma | MDA-1936-T |
| | | | 1424 | T | Adenocarcinoma | MDA-1424-T2 |
| | | | 2245 | T | Adenocarcinoma | MDA-2245-T |
| | | | 2327 | T | Adenocarcinoma | MDA-2327-T |
| | | | 1303 | T | Adenocarcinoma | MDA-1303-T2 |
| | | | 1216 | T | Adenocarcinoma | MDA-1216-T |
| | | | 1442 | T | Adenocarcinoma | MDA-1442-T |
| | | | 1974 | T | Adenocarcinoma | MDA-1974-T |
| | | | 1914 | T | Adenocarcinoma | MDA-1914-T |
| | | | 1366 | N | Squamous | MDA-1366-N |
| | | | 2119 | N | Squamous | MDA-2119-N |
| | | | 2155 | N | Squamous | MDA-2155-N |
| | | | 1358 | T | Squamous | MDA-1358-T |
| | | | 1642 | T | Squamous | MDA-1642-T |
| | | | 1012 | T | Squamous | MDA-1012-T |
| | | | 2014 | T | Squamous | MDA-2014-T |
| | | | 2285 | T | Squamous | MDA-2285-T |
| | | | 2262 | T | Squamous | MDA-2262-T |
| | | | 2324 | T | Squamous | MDA-2324-T |
| | | | 1517 | T | Squamous | MDA-1517-T |
| | | | 1579 | T | Squamous | MDA-1579-T |
| | | | 1366 | T | Squamous | MDA-1366-T |
| | | | 2264 | T | Squamous | MDA-2264-T |
| | | | 1640 | T | Squamous | MDA-1640-T |
| | | | 2119 | T | Squamous | MDA-2119-T |
| | | | 1652 | T | Squamous | MDA-1652-T |
| | | | 2155 | T | Squamous | MDA-2155-T |
| | | | 1692 | T | Squamous | MDA-1692-T |
| | | | 1699 | T | Squamous | MDA-1699-T |
| | | | 1150 | T | Squamous | MDA-1150-T |
| | | | 1555 | T | Squamous | MDA-1555-T |

| Sample | Experiment | Resource | Case ID (IW) | Tissue (Tumor or Normal) | Diagnosis | Sample ID (IW) |
|---|---|---|---|---|---|---|
| RNA | qPCR | MDAC | 2305 | N | Adenocarcinoma | MDA-2305-N |
| | | | 2309 | N | Adenocarcinoma | MDA-2309-N |
| | | | 2317 | N | Adenocarcinoma | MDA-2317-N |
| | | | 2318 | N | Adenocarcinoma | MDA-2318-N |
| | | | 2326 | N | Adenocarcinoma | MDA-2326-N |
| | | | 1193 | N | Adenocarcinoma | MDA-1193-N |
| | | | 1487 | N | Adenocarcinoma | MDA-1487-N |
| | | | 2188 | N | Adenocarcinoma | MDA-2188-N |
| | | | 2004 | N | Adenocarcinoma | MDA-2004-N |
| | | | 2037 | N | Adenocarcinoma | MDA-2037-N |
| | | | 2181 | N | Adenocarcinoma | MDA-2181-N |
| | | | 1994 | N | Adenocarcinoma | MDA-1994-N |
| | | | 2065 | N | Adenocarcinoma | MDA-2065-N |
| | | | 2243 | N | Adenocarcinoma | MDA-2243-N |
| | | | 2271 | N | Adenocarcinoma | MDA-2271-N |
| | | | 1914 | T | Adenocarcinoma | MDA-1914-T |
| | | | 2081 | T | Adenocarcinoma | MDA-2081-T |
| | | | 2327 | T | Adenocarcinoma | MDA-2327-T |
| | | | 1168 | T | Adenocarcinoma | MDA-1168-T |
| | | | 1186 | T | Adenocarcinoma | MDA-1186-T |
| | | | 1193 | T | Adenocarcinoma | MDA-1193-T |
| | | | 2309 | T | Adenocarcinoma | MDA-2309-T |
| | | | 1797 | T | Adenocarcinoma | MDA-1797-T |
| | | | 2230 | T | Adenocarcinoma | MDA-2230-T |
| | | | 1550 | T | Adenocarcinoma | MDA-1550-T |
| | | | 1303 | T | Adenocarcinoma | MDA-1303-T2 |
| | | | 1451 | T | Adenocarcinoma | MDA-1451-T2 |
| | | | 2318 | T | Adenocarcinoma | MDA-2318-T |
| | | | 1216 | T | Adenocarcinoma | MDA-1216-T |
| | | | 1769 | T | Adenocarcinoma | MDA-1769-T |
| | | | 1240 | T | Adenocarcinoma | MDA-1240-T |
| | | | 2102 | T | Adenocarcinoma | MDA-2102-T |
| | | | 1625 | T | Adenocarcinoma | MDA-1625-T |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | 1522 | T | Adenocarcinoma | MDA-1522-T |
| | | | 2245 | T | Adenocarcinoma | MDA-2245-T |
| | | | 1414 | N | Squamous | MDA-1414-N3 |
| | | | 1570 | N | Squamous | MDA-1570-N |
| | | | 1772 | N | Squamous | MDA-1772-N |
| | | | 2246 | N | Squamous | MDA-2246-N |
| | | | 1692 | N | Squamous | MDA-1692-N |
| | | | 1570 | T | Squamous | MDA-1570-T |
| | | | 2262 | T | Squamous | MDA-2262-T |
| | | | 1772 | T | Squamous | MDA-1772-T |
| | | | 2246 | T | Squamous | MDA-2246-T |
| | | | 1805 | T | Squamous | MDA-1805-T |
| | | | 1286 | T | Squamous | MDA-1286-T2 |
| | | | 2119 | T | Squamous | MDA-2119-T |
| | | | 1415 | T | Squamous | MDA-1415-T2 |
| | | | 1600 | T | Squamous | MDA-1600-T1 |
| | | | 1652 | T | Squamous | MDA-1652-T |
| | | | 1206 | T | Squamous | MDA-1206-T |
| | | | 1170 | T | Squamous | MDA-1170-T |
| | | | 1223 | T | Squamous | MDA-1223-T |
| | | | 1406 | T | Squamous | MDA-1406-T2 |
| | | | 1325 | T | Squamous | MDA-1325-T2 |
| | | | 1592 | T | Squamous | MDA-1592-T2 |
| | | | 1922 | T | Squamous | MDA-1922-T |
| | | | 1366 | T | Squamous | MDA-1366-T |
| | | | 1692 | T | Squamous | MDA-1692-T |
| | | | 1854 | T | Squamous | MDA-1854-T |

MDAC: M. D. Anderson Cancer Center

| Sample | Experiment | Resource | Sample ID (IW) | Stage |
|---|---|---|---|---|
| Genomic DNA from FFPE | qMSP | Aiichi Cancer Center | 1306805 | Non maligant |
| | | | 1306463 | Non maligant |
| | | | 1400206 | Non maligant |
| | | | 1400998 | Non maligant |
| | | | 1400046 | Non maligant |
| | | | 1400078 | Non maligant |
| | | | 1400026 | Non maligant |
| | | | 1400308 | Non maligant |
| | | | 1400369 | Non maligant |
| | | | 1400429 | Non maligant |
| | | | 1400691 | Non maligant |
| | | | 1400944 | Non maligant |
| | | | 1401020 | Non maligant |
| | | | 1203190 | AAH |
| | | | 1203217 | AAH |
| | | | 1301456 | AAH |
| | | | 1306463 | AAH |
| | | | 1306805 | AAH |
| | | | 1308120 | AAH |
| | | | 1203190 | AIS |
| | | | 1303636 | AIS |
| | | | 1305239 | AIS |
| | | | 1305576 | AIS |
| | | | 1305576 | AIS |
| | | | 1401497 | AIS |
| | | | 1203217 | MIA |
| | | | 1301456 | MIA |
| | | | 1306724 | MIA |
| | | | 1306945 | MIA |
| | | | 1307770 | MIA |
| | | | 1307843 | MIA |
| | | | 1308120 | MIA |
| | | | 1400630 | MIA |
| | | | 1401954 | MIA |
| | | | 1402519 | MIA |
| | | | 1400109 | ADC |
| | | | 1400121 | ADC |
| | | | 1400487 | ADC |
| | | | 1400713 | ADC |
| | | | 1401088 | ADC |
| | | | 1401166 | ADC |
| | | | 1401355 | ADC |
| | | | 1401459 | ADC |
| | | | 1401651 | ADC |
| | | | 1401810 | ADC |
| | | Vancouver General Hospital | | HYP |
| | | | | HYP |
| | | | | HYP |
| | | | | HYP |
| | | | | DYS |
| | | | | DYS |
| | | | | CIS |
| | | | | CIS |

FFPE: formalin fixed paraffin embedded

AAH:atypical adenomatous hyperplasia, AIS: adenocarcinoma in situ, MIA: microinvasive adenocarcinoma, ADC: invasive adenocarcinoma (ADC)

**Supplementary Materials & Methods Table S3. Primers list**

| **Primer list for bi-sulfite sequencing** | |
|---|---|
| Fragment-1-F | 5'-GAGATTTTGTTTTAGTGGAGGG-3' |
| Fragment-1-R | 5'-AAACTCCCTAAAAACCRACAT-3' |
| Fragment-2-F | 5'-GGTTTTTAGGGAGTTTTAAGG-3' |
| Fragment-2-R | 5'-ACCTTAATCAACTCCTCCTCTA-3' |
| Fragment-3-F | 5'-AGAGGAGGAGTTGATTAAGG-3' |
| Fragment-3-R | 5'-ATACCTCCCAATTACAAAAAA-3' |
| Fragment-4-F | 5'-GGGTATTAAGGTGAGGTAGG-3' |
| Fragment-4-F | 5'-ACACCTCAAAATTTAAAATCC-3' |

| **Primer list for chromatin IP** | |
|---|---|
| ITPKA promoter-F | 5'-GAGTCACCGAGAGCCTTCTC-3' |
| ITPKA promoter-R | 5'-CCGGGCAGGGTCATTTCC-3' |
| ITPKA body methylation region-1-F | 5'-AACCACTGGCAGAAGATCCG-3' |
| ITPKA body methylation region-1-R | 5'-CCCATTTTGCAGTCGAGCAC-3' |
| ITPKA body methylation region-2-F | 5'-GAAAATGCTGGCGGTGGATC-3' |
| ITPKA body methylation region-2-R | 5'-CGCGTAGTCTTGAAGTCGGT-3' |

| **Primer & probe list for qMSP analysis** | |
|---|---|
| Forward | 5'-GTAGGATTTGTTCGATGGTTTC-3' |
| Reverse | 5'-ACAAAACACGCATACCTAACG-3' |
| Probe | 5'-FAM CGGATTTTGTGTGTTCGATTGTAAAATGGG BHQ-1-3' |