Promoter Methylation Profiles between Human Lung Adenocarcinoma Multidrug Resistant A549/Cisplatin (A549/DDP) Cells and Its Progenitor A549 Cells

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Although aberrant DNA methylation has been implicated in the pathophysiology of lung cancer, the role of methylation in multidrug resistance (MDR) of lung cancer has remained unclear. To investigate whether certain distinct DNA methylation pattern is associated with acquired MDR of lung adenocarcinoma, methylated-DNA immunoprecipitation-chromatin immunoprecipitation (MeDIP-ChIP) was utilised to compare the genome-wide promoter methylation of the human lung adenocarcinoma MDR A549/cisplatin (A549/DDP) cells with its progenitor A549 cells. The comparison identified 3617 genes with differentially methylated promoter, of which 1581 were hypermethylated and 2036 were hypomethylated. Then, bisulphite sequencing polymerase chain reaction (BSP) and quantitative reverse transcription (RT)-PCR (Q-PCR) were used to validate the promoter methylation of five candidate genes and to determine whether the expression of genes was associated with the promoter methylation. BSP confirmed that the promoter methylation incidence of the hypermethylated genes, G protein-coupled receptor 56 isoform 3 (GPR56), metallothionein 1G (MT1G), and RAS association domain family gene 1 (RASSF1), was significantly higher in A549/DDP cells compared with A549 cells (p < 0.001, p = 0.0099, and p = 0.0165), whereas no significant difference was found in that of the other two genes, CCNL2 and BAD (p = 0.0594 and p = 0.5546). Additionally, Q-PCR showed that the mRNA expression of the three hypermethylated genes was significantly lower in A549/DDP cells compared with A549 cells (all p < 0.001). In conclusion, this study reported for the first time that a distinct promoter methylation pattern is associated with MDR of lung adenocarcinoma A549/DDP cells and suggested that GPR56, MT1G, and RASSF1 might be the potential methylation markers associated with acquired MDR of lung adenocarcinoma.

Key words: lung adenocarcinoma; multidrug resistance; DNA methylation; promoter

Lung cancer has replaced liver cancer to become the leading cause of cancer-related deaths in China, accounting for 22.7% of all cancer deaths. The rates of morbidity and mortality continue to rise rapidly and the lung cancer patients will reach one million in 2025 if no effective control measures were taken. Currently, lung adenocarcinoma has become the major pathologic type of lung cancer, the incidence of which accounts for 30 to 40% of all lung cancer cases in China and excess 50% in many areas of Europe. Chemotherapy is the major barrier to the successful chemotherapy treatment. Hence, understanding of the potential MDR mechanisms of lung adenocarcinoma is essential to discover novel chemotherapy drugs and improve the efficacy of chemotherapy treatment.

So far, most of MDR studies have been focused on the single genes to investigate the MDR mechanism of lung adenocarcinoma and obtained many important results. However, these chemotherapy resistance models are demonstrated to be relatively simplistic with the generally poor clinical outcomes. Interestingly, the growing evidences of epigenetic alterations in gene expression have provided an indication that DNA methylation status of a series of genes changed either simultaneously or sequentially might be involved in the chemotherapy-resistant phenotype of lung adenocarcinoma. First, many studies showed that DNA methylation is far more vulnerable than the DNA sequence to external factors. These alterations might be the earliest event in tumourigenesis, which could lead to a growth advantage for tumour cells and influence the direction of transformation. Second, DNA methylation changes can occur rapidly, which results in resistance arising quickly following chemotherapy treatment. Furthermore, the expression of multiple genes could be simultaneously affected by DNA methylation. Therefore, DNA methylation could be the driving force in acquired MDR, which have been confirmed in resistant tumour cell line of breast adenocarcinoma, murine neuroblastoma cells, and drug-resistant ovarian and colon tumour xenografts. These findings gave us clue that the DNA methylation might also play important roles in the development of the MDR polygenic phenotype of lung adenocarcinoma cells.

Until now, except a limited number of genomic regions or genes found to be methylated in MDR of lung cancer, few studies have reported a genome-wide promoter methylation patterns between human lung adenocarcinoma MDR A549/DDP cells and its progenitor A549 cells.
analysis to investigate whether certain distinct promoter methylation pattern is associated with MDR of lung adenocarcinoma. Thus, in the present study, to investigate the relationship between DNA methylation and MDR of lung adenocarcinoma, methylated-DNA immunoprecipitation–chromatin immunoprecipitation (MeDIP-ChIP) was utilised to compare the promoter methylation profiles of the human lung adenocarcinoma MDR A549/DDP cells with its progenitor A549 cells. Then, bisulphite sequencing polymerase chain reaction (PCR) (BSP) was performed to validate the results obtained from MeDIP-ChIP and quantitative reverse transcription (RT)–PCR (Q-PCR) was carried out to investigate whether the expression of the hypomethylated/hypomethylated genes was associated with the promoter methylation. The study preliminarily established a distinct DNA methylation pattern of the lung adenocarcinoma MDR cells and revealed several epigenetically inactivated genes, which might be the potential candidate genes or methylation markers involved in the MDR of lung adenocarcinoma.

MATERIALS AND METHODS

Cell Lines and Cell Culture  The MDR cell line A549/DDP was established as described as Guo. Briefly, the progenitor A549 cells were first treated with a high-dose shock of cisplatin (DDP) (1.0 µg/mL) and then stepwise selected for more than 6 months with increasing concentrations of cisplatin at a range of 0.05 to 1.0 µg/mL in RPMI-1640 medium (HyClone, Logan, UT, U.S.A.) with 10% foetal calf serum (Gibco, NY, U.S.A.) in a 37°C humidified incubator supplied with 5% CO₂. Then, the selected cells that demonstrated cross-resistance to hydroxy camptothecin, vincristine, and 5-fluorouracil (MDR A549/DDP) and A549 cells were regularly maintained in RPMI-1640 medium supplemented with 10% foetal calf serum in a 37°C humidified incubator supplied with 5% CO₂.

MeDIP-ChIP  MeDIP was performed as previously described. Briefly, genomic DNA from A549/DDP cells and A549 cells were extracted using QIAamp DNA Blood Mini Kit according to the manufacturer’s recommendation (QIAGEN, Maryland, U.S.A.), respectively, and fragmented by Bioruptor (Diagenode, Belgium). Immunoprecipitation of methylated DNA was performed using anti-5-methyl cytidine (mouse) and Biomag™ magnetic beads coupled anti-mouse immunoglobulin G (IgG). After the immunoprecipitated DNA was eluted and purified by phenol chloroform extraction, Input and IP DNA were labelled with Cy5- and Cy3-labeled random 9-mers, respectively, and hybridised to NimbleGen human genome annotations (HG18) RefSeq promoter arrays (Roche NimbleGen, Madison, WI, U.S.A.), which is single array design containing all known well-characterized 18028 RefSeq promoter regions (from about −2200 to +500 bp of the transcription start sites (TSSs)) totally covered by ca. 385000 probes. Triplicate sets of hybridisation were performed from three independent MeDIP experiments for each cell line. Finally, scanning was performed using a GenePix 4000B Microarray Scanner (Axon Instruments, Union City, CA, U.S.A.).

Tiling Array Data Analysis  The raw data were extracted as pair files by NimbleScan software. Then, a modified algorithm for capturing microarray enrichment (ACME) algorithm is employed where a fixed-length window (750 bp) is slid along the length of each chromosome, testing at each probe using a one-sided Kolmogorov–Smirnov (KS) test whether the surrounding window is enriched for high-intensity probes relative to the rest of the array. Each probe has a corresponding p-value score (−log10) and a threshold with positive signal difference is set to select regions that are enriched (i.e., methylated) in the test sample. Thus, transfrags or differentially methylated regions (DMRs) were generated by interval analysis with a p-value minimum threshold of 2, maximum spacing between nearby probes within a peak of 500 bp. When we get the DMRs data, we map them with genomic transcripts and make comparison analysis between 2 samples using NimbleGen SignalMap. Annotations of RefSeq were retrieved from the NCBI website. The correlation of log2 MeDIP/Input DNA ratios between replicates were computed using values from MaxTen calculations as described previously.

Promoter Methylation Analysis by BSP  Genomic bisulphite sequencing was performed to confirm the sensitivity of the observed DMRs. The genomic DNA was extracted from the A549 and A549/DDP cells using QIAamp DNA Blood Mini Kit according to the manufacturer’s recommendation (QIAGEN, Maryland, U.S.A.), respectively, and 400 ng genomic DNA was treated with sodium bisulphite using EZ DNA Methylation-Gold Kit (Zymo Research, Orange, CA, U.S.A.). Eighty to one hundred nanograms bisulphite-treated DNA was used for PCR amplification. The target regions of the relevant gene promoters and the primers, which were designed using the MethPrimer programme (http://www.urogene.org/methprimer/index1.html), were shown in Table 1. Then, the BSP products were cloned into a pMD®18-T vector according to the manufacturer’s instructions (TaKaRa, Dalian, China). For each cell line and each gene, five positive clones were randomly selected for subsequent sequencing. After this, the amplicon sequence data were aligned to the human reference genome, and the extent of methylation (DNA methylation levels) was determined by comparing the total number of Cs (methylated) to Ts (unmethylated) for each CpG site.

Gene Expression by Q-PCR  Total RNA was isolated using TRIzol according to the manufacturer’s recommendation (Invitrogen, Carlsbad, CA, U.S.A.). Two milligrams aliquots were reverse transcribed using an AMV First Strand cDNA Synthesis Kit according to the manufacturer’s instructions (Roche Applied Science, Mannheim, Germany). The SYBR green-based Q-PCR was then performed in triplicate using an ABI StepOnePlus Real-Time PCR Systems (Applied Biosystems, Foster, CA, U.S.A.) and the the level of gene expression was normalised by glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers for Q-PCR were listed in Table 2. PCR cycling conditions consisted of 5 min at 95°C followed by 40 cycles of 15 s of denaturation at 95°C, 30 s of annealing at 55°C and 30 s of extension at 72°C. The relative expression values were computed by the ΔΔCt method.

Statistical Analysis  Analysis for MeDIP-ChIP was performed using the ACME algorithm as previously described. Analysis for BSP was performed using chi-square test fourfold table. The expression of the five candidate genes and GAPDH in A549/DDP and A549 cells determined by Q-PCR was analysed by two-tailed Student’s t-test. p<0.05 was considered statistically significant.

RESULTS

Identification of Differentially Methylated Genes in
The comprehensive promoter methylation profiles were compared between A549/DDP and A549 cells using MeDIP-ChIP. The comparison identified 3617 genes with differentially methylated promoters in A549/DDP cells compared with A549 cells (1581 hypermethylated and 2036 hypomethylated). Global differential methylation analysis revealed that the genes with differentially methylated promoters were not evenly distributed across the genome. We observed that certain chromosomal were preferentially methylated or demethylated. For example, chromosomes 1, 2, 16, 17, and 19 were found to be intensively hypermethylated, chromosomes 1, 2, 3, 7, 9, 11, 12, 16, 17, 19, and X were found to be more hypomethylated (>100 genes), whereas chromosomes 13, 18, 21, and Y exhibited fewer differentially methylated genes (Fig. 1). The online GO analysis (http://gostat.wehi.edu.au/) categorized these genes with dif-

A549/DDP Cells Compared with A549 Cells The comprehensive promoter methylation profiles were compared between A549/DDP and A549 cells using MeDIP-ChIP. The comparison identified 3617 genes with differentially methylated promoters in A549/DDP cells compared with A549 cells (1581 hypermethylated and 2036 hypomethylated). Global differential methylation analysis revealed that the genes with differentially methylated promoters were not evenly distributed across the genome. We observed that certain chromosomal were preferentially methylated or demethylated. For example, chromosomes 1, 2, 16, 17, and 19 were found to be intensively hypermethylated, chromosomes 1, 2, 3, 7, 9, 11, 12, 16, 17, 19, and X were found to be more hypomethylated (>100 genes), whereas chromosomes 13, 18, 21, and Y exhibited fewer differentially methylated genes (Fig. 1). The online GO analysis (http://gostat.wehi.edu.au/) categorized these genes with dif-

### Table 1. Genomic Location of Five Candidate Genes with Hypermethylated or Hypomethylated Promoter for BSP Analysis

<table>
<thead>
<tr>
<th>GenBank No.</th>
<th>Genes</th>
<th>Location (HG18)</th>
<th>Distance from TSS</th>
<th>CpG sites in assay</th>
<th>Primers (5′→3′)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_005682</td>
<td>GPR56</td>
<td>Chr16</td>
<td>+98 to +495</td>
<td>9</td>
<td>TAGTATGTTGTTGTTAGGA</td>
<td>398</td>
</tr>
<tr>
<td>NM_005950</td>
<td>MT1G</td>
<td>Chr16</td>
<td>+82 to +309</td>
<td>6</td>
<td>CAAAACCAACAAACCAATC</td>
<td>228</td>
</tr>
<tr>
<td>NM_170714</td>
<td>RASSF1A</td>
<td>Chr3</td>
<td>+25 to +404</td>
<td>36</td>
<td>ACCCACTACCTCTCCCTTTC</td>
<td>380</td>
</tr>
<tr>
<td>NM_030937</td>
<td>CCNL2</td>
<td>Chr1</td>
<td>−2039 to −1703</td>
<td>9</td>
<td>CCTAACCAAAAAATTTCATTAC</td>
<td>337</td>
</tr>
<tr>
<td>NM_032989</td>
<td>BAD</td>
<td>Chr11</td>
<td>−1988 to −1642</td>
<td>8</td>
<td>TTAGGAGGAGGGTTGTAG</td>
<td>347</td>
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</table>

### Table 2. Primers Used for Q-PCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Sense (5′→3′)</th>
<th>Antisense (5′→3′)</th>
<th>Size (bp)</th>
</tr>
</thead>
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<tr>
<td>GAPDH</td>
<td>GGTGTCCTCTCTGACTCTCAACA</td>
<td>CCACCCACCTGTTGCTGTAG</td>
<td>136</td>
</tr>
<tr>
<td>GPR56</td>
<td>AAAGTACCAACCTACCGGA</td>
<td>GTCCTAAGTGGGGTCCCTCAAC</td>
<td>108</td>
</tr>
<tr>
<td>MT1G</td>
<td>TGTGGGGCTGTGCGGATGCTGTA</td>
<td>TTACGGGTCCTCTATTTGACTTG</td>
<td>118</td>
</tr>
<tr>
<td>RASSF1</td>
<td>GAGTACAAATGGCCAGATCAACA</td>
<td>AGGTTTGCTTTGCTTGGGA</td>
<td>130</td>
</tr>
<tr>
<td>CCNL2</td>
<td>CCACCTGGCTCTCAAGATAGA</td>
<td>AACCCACCACTCTTGGAGAACTCGT</td>
<td>182</td>
</tr>
<tr>
<td>BAD</td>
<td>GAGGATGAGTGACAGTTTGTG</td>
<td>GATCACCAGGACTCGGAAG</td>
<td>125</td>
</tr>
</tbody>
</table>

Fig. 1. The Distribution of Genes with Differentially Methylated Promoters along the Chromosomes in A549/DDP Cells Compared with A549 Cells (A) Distribution of genes with hypermethylated promoters. (B) Distribution of genes with hypomethylated promoters.
differentially methylated promoters into different biological functions, including transcription factor activity, plasma membrane part, sequence-specific DNA binding, transcription regulator activity, intrinsic to plasma membrane, ion transport, integral to plasma membrane, embryonic morphogenesis, gastrulation (Bonferroni \(p<0.05\)), and a series of signalling pathways with \(p<0.05\), including Wnt signalling pathway, tight junction, adherens junction, the TGF-beta signalling pathway, and others (Bonferroni \(p<0.05\)) (Fig. 2, Tables 3–4, supplementary Table 1–2). However, no significantly methylated promoters of some previously reported MDR genes such as \(MDR-1, LPR, MRP\) of lung cancer were detected in the present study.

**Validation of the Promoter Methylation in Five Candidate Genes** To validate the results obtained from the MeDIP-ChIP analysis, five differentially methylated promoter-associated genes, three of which were hypermethylated (\(GPR56, MTIG, and RASSF1\)) and two of which were hypomethylated (\(CCNL2 and BAD\)), were selected for BSP due to their special roles in tumorigenesis, tumour growth, or stress reaction. BSP results confirmed significantly increased promoter methylation of \(CCNL2, MT1G, and RASSF1\)) and two of which were hypomethylated (\(RASSF1\)) and two of which were hypomethylated (\(BAD\)).

### Table 4. Signal Pathways Involved in Genes with Hypomethylated Promoter Identified by MeDIP-ChIP Analysis in Lung Adenocarcinoma MDR A549/DDP Cells Compared with Its Progenitor A549 Cells

<table>
<thead>
<tr>
<th>Signal pathway</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroactive ligand-receptor interaction</td>
<td>CALC, DRD1, Tspo, THR, GABBR2, GRK2, NPY, DRD5, DRD5, NFKB1, GABBR1, GRIN3B, GABBR2, VIP1R, P2RY8, S1P3R, AGTR1, P2RY6, KISS1R, HRH3, P2RY1, S1P3R, GABRQ, HTR1E, GHR, GABRA2, PTGER3, CCKBR, GRIA4, GRM7, NPY, GRIN1, CRHR1, SST4R, CRHR2, SST2R, PRLR, PTGER5, HTR6, GPR50, UT52R, HTR2C, RDR1A1D, F2R</td>
</tr>
<tr>
<td>Glycerolipid metabolism</td>
<td>DGKA, CEL, AGPAT6, DGAT1, PPA2P2C, DGKG, LIPG, DGKZ, AGPA4T4, AGK, AGPAT3, AGPAT2</td>
</tr>
<tr>
<td>Regulation of actin cytoskeleton</td>
<td>FGF19, FGR2F, FGFR1, ENA, FGR3, MYL5, FGFI1, INSRR, ACTG1, PK7, PK3, GSN, CSK, TMSL3, GIT1, VAV3, ROCK1, LIMK1, MAP2K2, MYLK3, MYL12B, MYH9, FGFI2, VAV1, PPA1CB, ARP3, CFI1, SCIN, DGFRAM, DGFRB, TMS3B, PIP4K2A, F2R</td>
</tr>
<tr>
<td>Heparan sulphate biosynthesis</td>
<td>B3GAT3, HSST3A1, B3GALT6, XYL1T, HSST2, HSST3, HSSTS3B1, GLCE</td>
</tr>
<tr>
<td>Fc gamma R-mediated phagocytosis</td>
<td>PRKCA, PLD2, VAV3, LYN, LIMK1, PPA2P2C, NCF1, VAV1, AMPH, JMDL7-PLAG24, DOCK2, PLCG1, GAB, GSN, ARPC5, CFI1, INPP5D</td>
</tr>
<tr>
<td>Glycerophospholipid metabolism</td>
<td>PLD2, PPA2P2C, LPLA1, PS, DGKA, GPDL1, JMDL7-PLAG24, AGPA6, DGKG, DGKZ, PHOSPOH1, AGPA4T4, AGPAT3, AGPAT2</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>FZD9, SMO, STK36, WNT11, PTCH2, HHIP, FZD2, AXIN2, WNT6, SHH, TCF7L1, DVL1</td>
</tr>
<tr>
<td>(Vibrio cholerae) infection</td>
<td>PRKCA, ATP6V1C2, TCRG1, ACTG1, PRKACG, MUC2, ATP6V1C2, PLCG1, PDMA, RKN1, PRKX, NTP6V1F</td>
</tr>
<tr>
<td>Neurotrophin signalling pathway</td>
<td>IRK1, MAP2K2, MAP1K1, FOXO3, BAD, PTNN11, MAGED1, NTRK3, BDNF, MAP3K5, CAMK4, PLCG1, RPS6KA2, NTRK1, TACC, YWHAAQ, CALM3, SHB3, SHC, CSK, NGF</td>
</tr>
<tr>
<td>Calcium signalling pathway</td>
<td>GNA15, DRD1, GNA11, DRD5, PRKX, PRKACG, ATR1, PDE1B, PRKCA, PTGER3, CCKBR, BST1, MYLK3, GRM1, CD38, PLCG1, P2RX1, CAMK4, HTR6, AVPR1B, DGFRAM, CALM3, DGFRB, HTR2C, ADRA1D, F2R, CACNA1B</td>
</tr>
<tr>
<td>Axon guidance</td>
<td>ROCK1, EFN3, IMS1, GNA11, PLEX3, DYSPL2, CXCL12, EPHB1, WK7, SEMA6A, SEMA5B, RGS3, EPAG, UNC5A, PK3, FYN, CFL1, SEMA4D, RHOD, UNC5C, NFATC2</td>
</tr>
</tbody>
</table>
and \( p = 0.0165 \), respectively) (Figs. 3A–C), while no difference of the promoter methylation level of \( CCNL2 \) and \( BAD \) was found between A549/DDP cells and A549 cells (\( p = 0.0594 \) and \( p = 0.5546 \), respectively) (Figs. 3D, E).

**Expression of Five Candidate Genes** Aberrant promoter methylation is usually linked to an altered chromosomal state and, thus, to transcriptional gene silencing. \(^{28}\) Therefore, Q-PCR was carried out to investigate whether the expression of the five candidate genes was regulated by the promoter methylation. Q-PCR analysis demonstrated that except \( BAD \) (\( p = 0.426 \)), the expression of the other four genes were all significantly downregulated in A549/DDP cells compared with A549 cells (all \( p < 0.001 \)) (Fig. 4).

**DISCUSSION**

MDR is one of the major clinical obstacles to the successful treatment of lung adenocarcinoma. It appears to be a polygenic phenomenon in which epigenetic-mediated changes might be the driving force leading to this phenotype.\(^{29}\) To investigate whether certain distinct DNA methylation patterns is associated with the MDR phenotype of lung adenocarcinoma, MeDIP-ChIP was utilised to compare the promoter methylation profiles of the human lung adenocarcinoma MDR A549/DDP cells with its parental A549 cells. Totally, the promoters of 3617 genes were found to be differentially methylated, of which 1581 were hypermethylated and 2036 were hypomethylated in A549/DDP cells compared with A549 cells. To verify the MeDIP-ChIP results and investigate the association the expression of genes with its promoter methylation status, five
potential methylation markers of lung adenocarcinoma MDR, three genes with hypermethylated promoter (GPR56, MT1G, and RASSF1) and two genes with hypomethylated promoter (CCNL2 and BAD) were selected due to their special role in tumorigenesis, tumour growth, or stress reaction, using BSP and Q-PCR, respectively. The results indicated that the promoter methylation of GPR56, MT1G, and RASSF1 by BSP was consistent with that of MeDIP-ChIP, accompanied with transcriptional downregulation. However, the promoter methylation level of CCNL2 and BAD decreased but did not reach statistical significance in A549/DDP cells compared with A549 cells. Then, anticipated transcriptional upregulation of the two genes was not observed. Instead, the expression of CCNL2 was lower in A549/DDP cells compared with A549 cells, and which of BAD was unchanged.

RASSF1 and MT1G, two of the hypermethylated genes, were previously found to be involved in lung cancer progression and diagnostic, especially for non-small-cell lung cancer (NSCLC), and regulated by promoter methylation.30,31) RASSF1A, one isoform of RASSF1, has been identified as a tumour suppressor gene whose inactivation has been implicated in the development of many cancers, especially lung cancer. Metallothionein 1G (MT1G) belongs to a class of metal-binding proteins (MTs) and participates in several cellular processes, such as metal ion stability, detoxification, oxidation, and damage resistance. G protein-coupled receptor 56 isoform 3 (GPR56) is an orphan G protein-coupled receptor that has been shown to be implicated in brain development and play various roles in endogenous cancer progression,32) and suppress tumour growth and metastasis in human melanoma cell line xenograft models.33) But their roles in MDR of lung cancer is still unclear.34,35) Hence, this is the first study to report that the promoter methylation of these three genes was significantly higher in MDR cells of lung adenocarcinoma compared with its progenitor cells and the expression of these three genes was downregulated by the promoter hypermethylation. Therefore, we deduced that the three genes might be involved in the development of acquired MDR of lung adenocarcinoma. The mechanisms need be further investigated.

Interestingly, in the present study, Cyclin L2 (CCNL2), of which no promoter methylation was found between MDR cells with its progenitor cells by BSP, was found to be downregulated. The distance between the mCpGs and the TSS might be one of the most important reasons to explain this phenomenon;60) the distance between the mCpGs and the TSS of CCNL2 is relatively longer (−1000 to −2200bp), whereas that of the three hypermethylated genes (RASSF1, MT1G, and GPR56) is relatively shorter (−200 to +500bp). The phenomenon also provides evidence that the expression of gene is not correlated with its promoter methylation.37–40)

There are several limitations in the present study. First, several genes (e.g., MDR-1) that had previously been reported to be methylated in MDR cells were not identified by the MeDIP-ChIP. One of the possible reasons is that the promoter regions of these genes might not be covered by the HG18 methylation array. Additionally, heterogeneity might generate certain deviations.41,42) Second, though the promoters of 3617 genes were found to be differentially methylated and three candidate genes (GPR56, MT1G, and RASSF1) with differentially methylated promoter might be the potential methylation markers associated with acquired MDR of lung adenocarcinoma, whether other genes or signalling pathways were involved in the acquired MDR of A549/DDP cells need further investigation.

In summary, to our knowledge, the present study reported for the first time the genome-wide analysis of promoter methylation in the human lung adenocarcinoma MDR A549/DDP cells. Additionally, GPR56, MT1G, and RASSF1, three genes with differentially methylated promoter, might be the potential methylation markers associated with acquired MDR of lung adenocarcinoma.

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