Lung cancer continues to be a huge burden on the health status of Chinese and people worldwide, representing 15% of the new cases of all cancers diagnosed in 2008 (1). Lung cancer includes a group of heterogeneous histological types in terms of clinicopathological features and is commonly classified into two major subgroups, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC comprises nearly 20% of lung cancer with different biological behavior, clinical characteristics, responsiveness to chemotherapy and prognosis of patients (2). The malignant cells exhibit scarce cytoplasm, molded nuclei and neuroendocrine (NE) differentiation, as evidenced by expressing a variety of NE substance. But SCLC is also different from carcinoid in aspects of carcinogenesis and pathogenesis. Although the radio-chemotherapy and surgical resection for SCLC have considerable improvement, the patients still have poor prognosis with cumulative survival rate of around 5% at 5 years (3). The latest advances in molecular biological technologies have found some mechanisms of malignant neoplasia. In brief, SCLC cells show the abilities of continuous proliferation, evasion of apoptosis, local invasion and remote metastasis, which is related to silence of tumor suppressor genes (TSGs). Genetic and epigenetic alteration both are responsible for inactivation of TSGs. Epigenetic aberration, one of the earliest and most common phenomena in human malignancies, could lead to abrogation of various TSGs. Studies about epigenetic alteration could be contributed to increasing the rate of early stage at diagnosis, and therefore, the patients with SCLC could benefit from higher early detection possibilities with good responsiveness to intervention and long-time survival.

Expression of TSGs is regulated by the gene promoters, while CpG islands are commonly seen in the upstream of gene promoter. The clusters of guanine and cytosine (GC) are ranged from 0.5 to 5 kbp, with high ratio of GC (60-70%) (4). Almost fifty percent of the genes have the so-called CpG islands in the entire genome. About eighty percent of the CpG islands are hyper-methylated and related to repetitive elements in the human DNA. The novel thing is that such CpG islands located at the region of gene promoters are usually un-methylated in normal cells (5). Methylation of CpG islands at their cytosine 5 position is characterized by gene, tissue and differentiation specificities. Nevertheless, the mechanisms of gene silence via promoter methylation in transcription level are not fully understood. Current literatures have reported involved mechanisms as
follow: (I) DNA methylation may impede expression of house
keep genes necessary for epigenetic reprogramming (6); (II) 
Methylated DNA are interacted with some regulatory proteins,
including methylated DNA binding proteins (MBPs), MBPs 
like methyl-CpG binding protein 2 (MeCP2), and histone 
deacetylases (HDACs) (7); (III) Histone methylation via 
DNA methyltransferases (DNMTs) have additional chromatin 
modifications and mediate transcriptional repression (8).

Individual tumor types have their characteristic pattern of 
acquired aberrant methylation. Although many genes inactivated 
by methylation have been found in NSCLC, differences in 
molecular and genetic pathogenesis between SCLC and NSCLC 
can not be ignored. Data about aberrant methylation of TSGs 
in SCLC are limited. Aberrant hyper-methylated genes could be 
prognostic factors for SCLC, which need to be identified. In the 
present review article, we summarize the epigenetic methylation 
of key TSGs in SCLC. In addition, we will also provide a brief 
overview of the potential of these alterations as tumor markers 
and therapeutic targets for SCLC.

TSGs silenced by methylation in SCLC

**Cell cycle control genes**

P16\(^{INK}\) and P14\(^{ARF}\), located on chromosome 9p21, both 
are transcripts of the cyclin-dependent kinase inhibitor 
2A (CDKN2A), which are silenced via methylated in 
multiple tumors (9-11). Mitsuo Sato et al. (12) had reported 
comprehensive hyper-methylation profile of the p14ARF and 
p16\(^{INK4a}\) genes in 12 SCLC cell lines. In SCLC, p16\(^{INK4a}\) was 
methylated in about 31-37% of primary or metastatic tumors 
tissues (11,13). The hyper-methylated promoter was found at 
the frequency of 20% in all promoters for primary tumors 
samples and 13% for metastatic tumor samples. In SCLC cell 
lines, methylated P16\(^{INK}\) gene was detected in all promoter in 
H1618 cells. P16\(^{INK}\) gene was unmethylated in H82 and H146 
cells (11). Whereas, P14\(^{ARF}\) was methylated at a low frequency 
of 6% (12). Repression of p14\(^{ARF}\) is mainly due to homozygous 
deletion or mutation instead of heterozygosity methylation. 
Even Mitsuo Sato et al. (12) found there was no methylated 
promoter of p14\(^{ARF}\) gene in 12 kinds of SCLC cell lines. Those 
results indicated that hyper-methylation of p14\(^{ARF}\) promoter 
is rare in SCLC. But promoter methylation is the predominant 
mechanism for P16\(^{INK}\) inactivation.

The RAS association domain family 1A (RASSF1A) (also 
known as NORE2A, 123F2, RDA32 and REH3P21) is a 
gatekeeper for G1/S cell cycle progression, which regulates 
diverse cellular functions (14). Silence of RASSF1A leads to 
immortal cells proliferation and impeding apoptosis, which can serve 
as a useful diagnostic marker of cancers. M. Sato et al. (13) had 
detected the methylation status of RASSF1A gene in SCLC cells. 
The bisulfite genomic sequencing analysis showed RASSF1A 
promoters were complete methylated in 10 of 12 SCLC cell 
lines and incomplete methylated in 1 of 12 SCLC cell lines. 
RASSF1A is homogeneous deletion only in one SCLC cell line: 
H740 cells. Although Loss of heterozygosy (LOH) at 3p21.3 
occurs in more than 90% of SCLCs, Dammann R et al. (15) had 
demonstrated the CpG islands around RASSF1A promoters, 
located at 3p21.3, were hyper-methylated in 22 of 28 primary 
SCLC tissues. Pelosi G et al. (16) also found the expression 
level of RASSF1A mRNA is closely correlated with methylation 
degree of promoters. Further analysis indicated RASSF1A hyper-
methylation enabled a highly sensibility and relative specificity 
between patients with and without SCLC (17). Methylated 
RASSF1A gene detection may be a promising molecular tool for 
 diagnosis of primary SCLC.

**Apoptotic genes**

Death associated protein kinase (DAPK) gene is a novel 
tumor suppressor gene, which transmits apoptotic signals and 
malignant transformation (18). DAPK induces cell apoptosis 
through extensive intracellular signaling pathways, including 
serine/threonine kinase signal pathway, tumor necrosis factor-a 
(TNF-a) signal pathway, tumor growth factor-β (TGF-β) signal 
pathway, Fas ligand signal pathway and caspase signal pathway. 
However, apoptosis is only one side of duality of cell death. 
Autophagy, type II cell death (type I being the apoptotic cell 
death), was long ignored by the majority of the scientists. It exists 
in all organisms, especially under the malnutrition condition. 
Expression of DAPK, a potent cell death protein, also triggers 
the type II cell death process with autophage characteristics 
such as autophage vesicles and autolysosomes in cytoplasm. In 
most of human tumors, down-regulated expression of DAPK 
was not due to DNA deletion, rearrangement of DNA or LOH, 
but due to the epigenetic silencing by aberrant methylation. 
Promoter methylation of DAPK was detected by Esteller M and 
his colleagues in lung cancer (11). The frequency of methylation 
is 16%. The loss of DAPK expression acts as a molecular 
switch involved in disfunction of cell death like apoptosis and 
autophagy, which leads to pathogenesis and carcinogenesis.

Apoptosis-associated speck-like protein containing a CA 
spase recruitment domains (ASC) gene, protein kinase C delta 
binding protein (PRKCDBP) gene and Wilms tumor 1 (WT1) 
gene both are involved in cell apoptosis via various cellular signal 
pathways. The expression of those genes in SCLC was blocked 
compared to normal lung (19). Fukasawa M et al. (19) found 
those gene both were hyper-methylated in SCLC cell lines using 
the promoter associated methylated DNA amplification and 
DNA chip (PMAD) method. Hyper-methylation of the ASC 
gene promoter was detected in LK79 cells, S2cells and SBC-3 
cells, PRKCDBP gene in S2cells and SBC-3 cells and WT1 gene
in LK79 cells and SBC-3 cells. The average hyper-methylation rate was 14.0% for SCLC (LK79, S-2, SBC-3 cells). Target of methylation induced silencing (TMS1) gene, also known as ASC gene, encodes a 22-kDa CA spase recruitment domains (CARD) protein, which acts as a adapter protein. This adapter protein activates caspases through cleavage of proteins pathway via CARD domains. Activated caspases stimulate a family of cysteine proteases and trigger programmed cell death. However, the ability of response to caspases can be lost due to epigenetic silence of TMS1 gene in SCLC cells (20). Methylation-specific polymerase chain reaction (MSP) test indicated that aberrant methylation of TMS1 was present in 70% (40 of 57) of SCLC cell lines and 41% (13 of 32) of SCLC tumor tissues (20).

Caspase-8 is a key component of apoptotic complexes. Fulda S et al. (21) reported the caspase-8 mRNA expression was in different levels in SCLCs. The expression levels of caspase-8 protein were associated with the methylation status of a CpG-rich part of the 5' flanking region of caspase-8. In VH-64 cells with a relative high level of caspase-8, only the unmethylated CpG islands of caspase-8 promoter were found. While in cell lines with undetectable levels of caspase-8 expression like CADO cells, CpG islands of caspase-8 were detected only in the methylated form.

**DNA repair genes**

DNA repair plays a critical role in the regulation of various physiological or pathological conditions and remove of mutagenic and cytotoxic production. O^6^-methylguanine DNA methyltransferase (MGMT) gene, encoding O^6^-AG DNA alkyltransferase, has also been implied to mediate DNA repair by remove of alkyl groups from the O^6^ position of guanine. Few scholars had reported epigenetic silencing of MGMT gene via hyper-methylation of promoter leads to loss of MGMT activity (11,12,24). Methylation of MGMT promoter was found in 36 of 39 (92%) SCLC cell lines (23). The frequency of methylated MGMT genes in SCLC tissues was 16% (24). However, studies of Esteller M (25) had opposite results. Promoter hyper-methylation of MGMT gene was a common event in NSCLC samples (29%). There was no epigenetic methylation in SCLC samples.

Fragile histidine triad (FHIT) gene is a DNA repair gene, which loss of expression is frequent in SCLC. In addition, the correlation between FHIT methylation and lack of FHIT expression was highly significant (P<0.0001) (26). The frequency of methylated FHIT gene is 64% in SCLC samples (24).

**Metastasis related genes**

Cadherin 1 (CDH1), cadherin 13 (CDH13), deleted in liver cancer 1 (DLC1) and tissue inhibitor of metalloproteinase 3 (TIMP3) both are classic TSGs and key cell adhesion molecules to maintain normal tissue architecture and inhibit tumor initiation, adhesion and metastasis. CDH1 gene was methylated in 17 of 39 (44%) SCLC cell lines (23), while methylation of CDH13 promoter was at a higher frequency of 92% (36 of 39). And other reports had different results. Zöchbauer-Müller S et al. reported the frequency of methylated CDH13 gene was only 15% (24). Fukasawa M et al. (19) found CDH13 was methylated in LK-79 and SBC-3 cells, but not in S2 cells. And their studies also indicated that methylated DLC1 gene was only found in SBC-3 cells. Esteller M and his colleagues (11) found the frequency of hyper-methylated TIMP-3 gene was 19% in lung cancer tissues.

**Other TSGs**

Similarly, other TSGs related to carcinogenesis silenced by promoter methylation, such as adenomatous polyposis coli (APC) (23,24,26), zinc finger (Blu) (27), calcitonin-related polypeptide alpha (CALCA) (19), epidermal growth factor like domain 7 (EGFL7) (15), endothelin B receptor (ETBR) (28), fatty acid binding protein 3 (FABP3) (19), GATA binding protein 4 (GATA4) (29), GATA binding protein 5 (GATA5) (29), glutathione S transferase pi 1 (GSTP1) (11,23,24), hyper-methylated in cancer 1 (HIC1) (19), S-hydroxytryptamine receptor 1B (HTR1B) (19), interferon regulatory factor 7 (IRF7) (19), low density lipoprotein-related protein 2 (LRP2) (19), myosin 18B (MYO18B) (30), myogenic differentiation 1 (MYOD1) (19), neuron-restrictive silencer factor (NRSF) (31), paired box gene 3 (PAX3) (19), receptor interacting serine threonine kinase 3 (RIPK3) (19), retinoic acid receptor beta (RAR-β) (19,23,24,32), Ras-related associated with diabetes (RRAD) (33), sex determining region Y box 18 (SOX18) (15) and thyroid hormone receptor β1 (TRβ1) (34) have already been identified in SCLC (Table 1).

Above results may cause considerable controversies about the degree and frequency of TSGs methylation in SCLC. The frequency of some TSGs methylation varies widely in different literatures. These studies reflect differences between primary SCLC tissues and cell lines. The degree of methylation also differ among cell lines with differentiation subtypes. Consistent with this, the compatibility, veracity and reliability of detected methods need to be demonstrated.

**Clinical application of methylated TSGs**

Analysis of methylation of TSGs has a huge application foreground with unparalleled advantages. First, DNA is easier to extract from blood, biological fluid, tumor mass and formaldehyde-fixed samples due to stability of DNA. Second, methylation-specific PCR has relatively high sensitivity and a
<table>
<thead>
<tr>
<th>Gene name</th>
<th>Full name</th>
<th>Location</th>
<th>Major functions</th>
<th>Ref.</th>
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<tr>
<td>APC</td>
<td>Adenomatous polyposis coli</td>
<td>5q21-q22</td>
<td>Chromosome segregation</td>
<td>(23,24,35)</td>
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<td>ASC</td>
<td>Apoptosis-associated speck-like protein containing a CARD</td>
<td>16p12-p11.2</td>
<td>Pro-apoptosis</td>
<td>(19,20)</td>
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<td>BLU</td>
<td>Zinc finger</td>
<td>3p21.3</td>
<td>Stress response</td>
<td>(27)</td>
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<td>CALCA</td>
<td>Calcitonin-related polypeptide alpha</td>
<td>11p15.2-p15.1</td>
<td>Inflammation</td>
<td>(19)</td>
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<tr>
<td>CASP8</td>
<td>Caspase 8</td>
<td>2q33-q34</td>
<td>Initiation of apoptosis</td>
<td>(21)</td>
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<td>CDH1</td>
<td>Cadherin 1/E-cadherin</td>
<td>16q22.1</td>
<td>Cell adhesion, proliferation</td>
<td>(23)</td>
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<td>(19,23,24)</td>
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<td>DAPK</td>
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<td>EGFL7</td>
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<td>9q34.3</td>
<td>NOTCH4-like</td>
<td>(15)</td>
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<td>ETBR</td>
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<td>13q22</td>
<td>G protein coupled receptor activity</td>
<td>(28)</td>
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<td>(24,26)</td>
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<td>GATA binding protein 5</td>
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<td>(29)</td>
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<td>GSTP1</td>
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<td>DNA repair</td>
<td>(11,22-24)</td>
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<td>(30)</td>
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<td>Hedgehog signaling pathway</td>
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<td>9p21</td>
<td>Stabilizing p53, cell cycle</td>
<td>(12)</td>
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<td>P16INKF</td>
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<td>Cell cycle</td>
<td>(11-13)</td>
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<td>Pro-apoptosis</td>
<td>(19)</td>
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<td>RARβ</td>
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<td>3p24</td>
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<td>Cell cycle, apoptosis</td>
<td>(13-17)</td>
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<td>16q22</td>
<td>GTPase signal pathway</td>
<td>(33)</td>
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<td>SOX18</td>
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<td>HMG-box transcription factor</td>
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<td>TIMP3</td>
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<td>22q12.3</td>
<td>Cell migration, differentiation</td>
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<td>TMS1</td>
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<td>Thyroid hormone receptor β1</td>
<td>3p24.2</td>
<td>ligand-dependent transcription factors</td>
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<td>Wilms tumor 1</td>
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<td>Pro-apoptosis</td>
<td>(19)</td>
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</table>
DNA-based methylated marker can be detected among one thousand unmethylated alleles (36). Third, methylated DNA test is a noninvasive diagnostic method. Fourth, methylation of TSGs occurs in early stage of SCLC, which can be used for screening. Furthermore, methylation of TSGs can be reserved by methylation reagent, like S-Aza-2'-deoxycytidine and its analogues (21,35) and TSGs methylation is correlated with response to chemotherapy (22).

According to the literature (17), methylated RASSF1A promoters were detected in 35 of 40 bronchial aspirates samples collected from patients with SCLC. However, RASSF1A hyper-methylation was not founded in 0 of 46 samples from patients with non-neoplastic lung disease. Moreover, the frequency of RASSF1A methylation increases along with poor differentiation of SCLC cells. Methylation of TSGs can be prognostic predictor for patient with SCLC. The 2-year survival for patients who were positive and negative for RAR-β methylation was significantly different in SCLC patients (chi-square test, P=0.044) (32). Reactivation of TSGs by S-Aza-2'-deoxycytidine might be an effective strategy to intervene in SCLC (21,35). Pietanza MC et al. (22) found patients with methylated MGMT had higher complete response rate or partial response rate compared to those with unmethylated MGMT (38% vs. 7%) to temozolomide. Restoration of sensitivity for chemotherapy is a useful approach to improve long-term survival of patients with SCLC.

Conclusions

SCLC pathogenesis is a multi-step and multi-gene controlled process, involved in both genetic and epigenetic mechanisms. Aberrant alternation of TSGs silenced by promoter methylation is crucial in progression of SCLC, which has not been given well-deserved focus. Recently, more and more methylated TSGs have been identified in SCLC and thus provide a new insight into the mechanisms of initiation and development of SCLC. Methylated TSGs also provide new tumor markers, intervening approaches and prognostic factors for patients with SCLC. However, some problems remain to be solved. The key point is that CpG islands are located at various positions, such as exons and introns, not confined in promoter region of the TSGs. Although 79% of CpG islands are located in the promoter and the first exon, others are outside of these regions (19). Fukasawa M et al. (19) had reported most of detected TSGs were hyper-methylated in SCLC by microarray of cDNA library using CpG island clones. So data of methylation profile in promoter region is limited. The criteria of hyper-methylation is still unclear. Most of scholars define hyper-methylation of TSGs as follow: the ratio of normalized methylation intensity of cancer tissues to normalized methylation intensity of normal tissues is more than 3.0. The ratio less than 3.0 or less than background intensity represents un-methylation of TSGs. With the use of genome-wide epigenomic approaches (37) and scientific definition of hyper-methylation, the liable methylated TSGs might improve the early screen, treatment and prognosis of SCLC in future.

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