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# Circulating epigenetic biomarkers in lung malignancies: From early diagnosis to therapy

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ABSTRACT

Lung cancer (LC) and malignant mesothelioma (MM) are malignancies linked to environmental/occupational exposure, which are increasing in incidence. Despite advances in chemotherapy, radiation therapy and surgical management of LC and MM, the median survival remains less than 12 months. Early detection represents one of the most promising approaches to reducing the growing cancer burden by increasing chemotherapy treatment efficiency. At present, early diagnosis is rather difficult and depends on invasive sampling of pleural fluid or tissue. Currently the most widely used screening method for the surveillance of exposed subjects is computed tomography (CT), which is costly, exposes patients to repeated high doses of radiation, and typically detects the malignancy at its advanced stage. Recently, a virtually non-invasive 'liquid biopsy' has emerged as source to characterize tumour heterogeneity. The genetic/epigenetic changes during tumour evolution can be detected in fluids and used as cancer biomarkers. Therefore, increasingly interest has been paid to circulating (cell-free) nucleic acids (cfDNA/cfmiRNAs) epigenetically modulated during cell transformation. Hypermethylation of tumour suppressor genes is frequently observed in cancers, and such epigenetic changes are potential markers for detecting and monitoring tumours. The same predictive biomarkers can be used as therapy targets.


## 1. Introduction

Lung cancer (LC) is growing worldwide, representing the second most common cancer and a leading cause of cancer-related deaths [1–3]. Similarly, the incidence of malignant mesothelioma (MM), albeit a rare type of cancer, is increasing as result of widespread exposure to asbestos [4]. Approximately 80% of MM patients have a history of occupational asbestos exposure, which is considered a risk factor for the development of the disease. The molecular pathogenesis of LC and MM is not well understood. Beside to the most common mutations found either in LC or in MM [5–7], epigenetic alterations, such as DNA methylation, have been found in tumours, where MM have a different profile compared with LC [8]. The epigenome is both dynamic and highly regulated in order to conserve normal expression patterns in cells. One of the most important components of expression regulation is methylation of CpG islands within promoter regions both in normal and cancer cells [9,10].

Alterations in epigenetic profiling may provide important insights into the aetiology and natural history of cancer. Since several epigenetic changes occur prior to histopathological changes, they can serve as biomarkers for cancer diagnosis and risk assessment. Many cancers may remain asymptomatic until relatively late stages. In managing the disease, effort ought to be focused on early detection, accurate prediction of disease progression, and frequent monitoring. The current gold standard of cancer diagnosis is based on histology evaluation of tissue biopsies. However, these techniques are invasive and expensive. The use of biological flu-

*Abbreviations:* MM, Malignant mesothelioma; LC, lung cancer; SMRPs, soluble mesothelin-related proteins; miRNA, microRNA; TM, thrombomodulin; EGFL7, EGF-like-domainmultiple 7; ROC, receiver operating characteristics; AUC, area under curve; CT, computed tomography; VEGF, vascular endothelial growth factor; NSCLC, non-small cell lung cancer; cfDNA, cell free DNA; SCC, squamous cell carcinoma; AD, adenocarcinoma; MSC, miRNA signature classified; MSLN, methylation status of mesothelin gene; DNMTi, DNA methyltransferase inhibitors; HDACi, histone deacetylase inhibitors; LDCT, low-dose computer tomography; EVs, extracellular vesicles; CDKN2A/ARF, cyclin-dependent kinase inhibitor 2A/alternative reading frame; NF2, neurofibromatosis type 2; BAP1, BRCA1-associated protein-1; RASSF1A, Ras-association domain family; CDKN2A, cyclin-dependent kinase inhibitor 2A; MGMT, O-6-methylguanine-DNA methyltransferase.

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ids defined as 'liquid biopsies', represent a source of non-invasive biomarkers for cancer [11]. In solid tumours, circulating biomarkers can be released into bloodstream through various events including necrosis, apoptosis, and other physiological mechanisms in the stromal microenvironment. Therefore, 'liquid biopsies' can capture spatial and temporal heterogeneity during tumour formation and evolution. Epigenetic aberrations offer dynamic and reversible targets for cancer therapy. Increasingly, alterations via overexpression, mutation, or rearrangement are found for genes that control the epigenome. This review describes epigenetic circulating biomarkers as they are expressed during cancer development and their potential use in lung malignancies diagnosis, prognosis, and treatment.

## 2. Epigenetics in lung malignancies

Lung cancer involves accumulation of genetic and epigenetic events in the respiratory epithelium [12]. Inactivation of tumour suppressor genes via promoter methylation, often referred to as hypermethylation, is a hallmark of lung cancer and is an early event in the carcinogenic process [13]. Promoter methylation can couple with mutation or deletion events to inactivate a tumour suppressor gene. Generally, inactivation of one allele is insufficient to lead to clonal selection. However, there is evidence indicating that, at some gene loci, both copies do not necessarily need to be inactivated to adversely impact the cell, partial inactivation of one allele contributing to carcinogenesis [14]. Various toxic/stress factors can modify mammalian cells resulting in an epigenetically transformed phenotype without changing the DNA sequence information of the cells; these include radiation, smoking, hormones, asbestos, arsenic, radon, nickel, reactive oxygen species (ROS) and various chemicals. Lung cancer is communally associated with tobacco smoke exposure. However, LC in 'never smokers' accounts for 13–28% of all cases [15]. Exposure to arsenic, radon, and asbestos is also involved in lung tumourigenesis [16–19]. These lung carcinogens, can induce a wide range of molecular alterations, both of genetic (point-mutations and genome-wide aberrations) and epigenetic nature, including changes in DNA methylation and miRNA expression. A scheme showing how a toxicant such as asbestos can induce DNA methylation in the site of the DNA damage is shown in Fig. 1.

Long-term radon exposure has been associated with epigenetic change such as increased cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation among Chinese miners [20]. Epigenetic inactivation of tumour-suppressor genes, such as RASSF1A and p16, has been observed in lung cancer patients exposed to asbestos [21,22]. Hypermethylation of *CDKN2A* may occur early in the genesis of lung cancer, having been identified in pre-malignant lesions [23]. Similarly, promoter methylation of Ras-association domain family (*RASSF1A*), *APC*, *ESR1*, *ABCB1*, *MT1 G* and *HOXC9* has been associated with stage I of non-small cell lung cancer (NSCLC) [24,25]. Conversely, other hyper-methylated genes, such as *hDAB2IP*, *H-cadherin*, *DAL-1*, and *FBN2*, have been related to advanced stage NSCLC [26–28], suggesting that these changes may occur at a later point in the carcinogenesis process. Epigenetic factors can be responsible for aberrations of the miRNome (defined as the full spectrum of miRNAs for a specific genome) observed in cancer. Indeed, miRNAs undergo the same epigenetic regulations like any other protein-coding gene. Moreover, a specific group of miRNAs (defined as *epi*-miRNAs) can directly target effectors of the epigenetic machinery (such as DNA methyltransferases, histone deacetylases, and polycomb repressive complex genes) and indirectly affect the expression of tumour suppressor genes, whose expression is controlled by epigenetic factors. A specific miRNA

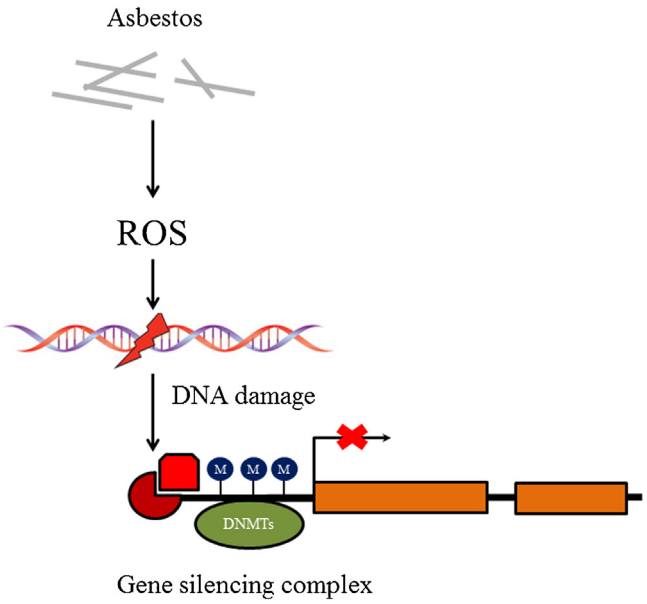


**Fig. 1.** Oxidative DNA damage and epigenetic changes. Asbestos-induced ROS formation promotes DNA damage (breaks, oxidised bases). Recruitment of gene silencing systems such as the DNA damage proteins (γ-H2AX, SIRT1) and DNA-methylating enzymes (DNMT1, DNMT3B), to sites of oxidative damage follows. The methylation of CpG promoter islands induces epigenetic gene silencing.

alteration signature in carcinogen-induced malignancies has been detected [29]. A recent study has identified an asbestos-associated miRNA signature in lung cancer, where miR-148b, mi*R*-374a, miR-24-1, Let-7d, Let-7e, miR-199b-5p, mi*R*-331-3p, and miR-96 were found to be over-expressed, while miR-939, miR-671-5p, miR-605, miR-1224-5p and miR-202 were 'under-expressed' [30].

Asbestos is known to be a mutagen, albeit weak, [31,32], and there are several reports on tumour suppressor gene methylation in MM [33,34]. Similar to other cancer, genesis of MM is associated with genomic mutations as well as epigenetic errors leading to modifications of gene expression [32]. Induction of methylation in a phenotypically important pathway occurs as a result of physical interaction between asbestos fibers and the parietal pleura. Prevalence of *RASSF1* methylation was found in MM (33%); it was also found that *RASSF1* methylation is significantly associated with increased asbestos body count, with prevalence of methylation at the *APC*, *CDKN2A* and *CDKN2 B* [35]. The cyclin-dependent kinase inhibitor 2A/alternative reading frame (*CDKN2A/ARF*), neurofibromatosis type 2 (*NF2*) and BRCA1-associated protein-1 (*BAP1*) genes are the most frequently mutated tumour suppressor genes detected in MM cells [36]. BAP1 is involved in histone modification and its inactivation induces the disturbance of global gene expression profiling. Methylation status of the promoter region of nine candidate genes has been reported [37]. High methylation frequency was found for *E-cadherin* (71.4%) and *FHIT* (78%), and to a lower extent *ACP1A* (14.3%), *RASSF1A* (19.5%), and *DARK* (20%). Intermediate values were observed for p16 (*INK4a*) (28.2%), *APC1 B* (35.5%), p14 (*ARF*) (44.2%) and *RAR-b* (55.8%). Deregulated miRNA profile has also been detected in MM [38–41]. These data thus indicate that promoter methylation associated with alterations of gene expression is a common event in MM.

## 3. Epigenetically regulated genes as biomarkers

Despite recent advantages in LC research and the use of novel therapeutic agents for NSCLC, the outcome remains dismal with 5-years survival rate of about 15% [3]. Currently, majority of lung cancers are detected at an advanced stage in which treatments