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Distinct DNA methylation profiles in malignant mesothelioma, lung adenocarcinoma, and non-tumor lung

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Summary DNA methylation markers provide a powerful tool to make diagnoses based on genetic material obtained directly from tumors or from ''remote'' locations such as sputum, pleural fluid, or serum. In particular when limited cell numbers are available, amplifyable DNA markers can provide a very sensitive tool for cancer detection and classification. Malignant mesothelioma (MM), an aggressive cancer strongly associated with asbestos exposure, can be difficult to distinguish from adenocarcinoma of the lung when limited material is available. In an attempt to identify molecular markers for MM and adenocarcinoma, we examined the DNA methylation status of 14 loci. Analysis of methylation levels in 10 MM and 8 adenocarcinoma cell lines showed that methylation of APC was significantly elevated in adenocarcinoma

Abbreviations: AD, lung adenocarcinoma; APC, adenomatosis polyposis coli; CALCA, calcitonin-related polypeptide α; CDH1, E-cadherin; CDKN2A, cyclin-dependent kinase inhibitor 2A; ESR1, estrogen receptor 1; ESR2, estrogen receptor 2; GSTP1, glutathione *S*-transferase π; MGMT, O-6-methylguanine-DNA methyltransferase; MM, malignant mesothelioma; MTHFR, 5,10-methylenetetrahydrofolate reductase; MYOD1, myogenic factor 3; NL, non-tumor lung; PGR, progesterone receptor; PMR, percentage methylated reference; PTGS2, prostaglandin G/H synthase; RASSF1, Ras association domain family; TIMP3, tissue inhibitor of metalloproteinase 3

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compared to MM cell lines ($P = 0.0003$), while methylation of CDH1 was higher in MM ($P < 0.02$). Subsequent examination of the methylation status of the 14 loci in 6 MM and 7 adenocarcinoma primary tumors, which yielded similar methylation profiles, supported these observations. Comparison of methylation in MM cell lines and tumors versus non-tumor lung tissue indicated that APC exhibits less methylation in MM ($P = 0.003$) while RASSF1, PGR1, ESR1, and CDH1 show more methylation in MM, the latter two showing the most significant difference between the two tissue types ($P \leq 0.0001$). Comparison of methylation in adenocarcinoma cell lines and tumors versus non-tumor lung tissue showed methylation of ESR1, PGR1 and RASSF1 to be significantly elevated in adenocarcinoma, with RASSF1 being most significant ($P = 0.0002$). Thus, with the examination of 14 loci, we have identified 5 candidates that show potential for distinguishing between MM, adenocarcinoma and/or non-cancer lung. Our observations support the strong potential of methylation markers as tools for accurate diagnosis of neoplasms in and around the lung.

1. Introduction

More than 2000 cases of malignant mesothelioma (MM) are diagnosed in the United States each year [1]. MM is an aggressive cancer, with death usually occurring within a year after diagnosis [1]. It is strongly associated with asbestos exposure and is thought to be derived from either the mesothelial serosal lining of the coelomic cavity or the subserosal mesenchymal cells that line the pleura and peritoneal cavity [2]. When MM arises in the pleura, it can be difficult to distinguish from adenocarcinoma of the lung, one form of which can grow diffusely over the pleura, in a manner similar to mesothelioma [3], in particular when scant material is available [1,4—6]. This can lead to delays in the administration of proper treatment. DNA-based molecular markers that specifically distinguish between lung adenocarcinoma and mesothelioma would be of great value because they require few if any intact tumor cells. While such markers could be used to confirm a histological diagnosis based on tumor samples, they show an even stronger promise as future tools to examine ''remote'' material (serum, sputum, pleural fluid) in at risk populations.

One emergent molecular marker that has shown significant promise for cancer diagnosis is DNA hypermethylation [7,8]. DNA methylation is essential for proper mammalian development and occurs at the 5 position of cytosine in a CpG dinucleotide context [9,10]. In normal cells, clusters of CpG dinucleotides, also known as CpG islands, are usually not methylated. However, in cancer cells, these CpG islands may become hypermethylated. CpG islands are often found in the promoter regions of genes and de novo methylation of these promoter CpG islands is associated with gene silencing [11]. The frequently observed methylation-mediated silencing of tumor suppressor genes and other genes involved in cellular growth regulation indicates that methylation plays an important role in carcinogenesis [12—14]. CpG island methylation analysis of a variety of cancers has suggested that cancers from different organs display distinct methylation profiles [15]. Even different histological subtypes of cancers within a given organ appear to have distinct methylation profiles. This is illustrated by the recent analysis of DNA methylation levels in 91 lung cancer cell lines: 7 out of the 23 CpG island loci tested showed statistically significant differences in the methylation values between small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) cell lines [16]. Thus, it seems likely that MM and lung adenocarcinoma might also be distinguished on the basis of their methylation profiles.

The primary objective of this study was to identify genes that show elevated methylation in MM and/or lung adenocarcinoma, with the goal of developing potential new methylation markers that might be used for diagnosis of these types of cancer, in particular in situations where material is scant. The first step in the lengthy process of marker development is the identification of candidate markers [17]. Although DNA methylation has been studied in many tumor types, MM has received relatively little attention. The methylation levels of only a handful of genes have been studied in mesothelioma [18—26]. Most studies evaluated individual genes, and a variety of qualitative or semi-quantitative techniques varying in sensitivity were used; therefore, results from different studies cannot necessarily be compared. Here, we describe the analysis of the largest panel of DNA methylation loci examined to date in MM. DNA methylation levels were simultaneously analyzed at 14 loci, using the highly sensitive, quantitative MethyLight