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DNA methylation profile of 28 potential marker loci in malignant mesothelioma

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Summary Patients with malignant mesothelioma (MM), an aggressive cancer associated with asbestos exposure, usually present clinically with advanced disease and this greatly reduces the likelihood of curative treatment. MM is difficult to diagnose without invasive techniques; the development of non-invasively detectable molecular markers would therefore be highly beneficial. DNA methylation changes in cancer cells provide powerful markers that are potentially detectable non-invasively in DNA shed into bodily fluids. Here we examined the methylation status of 28 loci in 52 MM tumors to investigate their potential as molecular markers for MM. To exclude candidate MM markers that might be positive in biopsies/pleural fluid due to contaminating surrounding non-tumor lung tissue/DNA, we also examined the methylation of these markers in lung samples (age- or environmentally induced hypermethylation is frequently observed in non-cancerous lung). Statistically significantly increased methylation in MM versus non-tumor lung samples was found for estrogen receptor 1 (ESR1; $p = 0.0002$), solute carrier family 6 member 20 (SLC6A20; $p = 0.0022$) and spleen tyrosine kinase (SYK; $p = 0.0003$).

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Examination of associations between methylation levels of the 28 loci and clinical parameters suggest associations of the methylation status of metallothionein genes with gender, histology, asbestos exposure, and lymph node involvement, and the methylation status of leucine zipper tumor suppressor 1 (LZTS1) and SLC6A20 with survival.

1. Introduction

Malignant mesothelioma (MM) is an aggressive cancer of the pleura, peritoneum or pericardium strongly associated with exposure to asbestos [1—3]. MM usually becomes clinically apparent after a 30—40-year latency period following asbestos exposure [4]. The use of asbestos in developed countries has continuously declined in the past 30 years and because of this, mesothelioma incidence is expected to peak in many countries. In the United States, the mesothelioma incidence peaked in the 1990s [5]. However, more than 2000 cases are still diagnosed annually and workers previously active in construction, railroads and shipyards will continue to be at risk for developing MM [4,6]. Recently, concerns were raised about asbestos exposure of New York residents and public servants such as firefighters, rescue workers, and recovery teams after the collapse of the World Trade Center on 11 September, 2001 [7]. In the United Kingdom, the peak of mesothelioma deaths is expected to fall around 2020 [3]. In contrast to the diminished use of asbestos in developed countries, asbestos use in Asian and Latin American nations has become a more common trend [3]. For this reason, it is projected that worldwide mesothelioma incidence will continue to increase in the next 10—20 years.

Because clinical symptoms only appear with advanced disease, the median survival for mesothelioma patients is approximately 9 months [8]. Mesothelioma is usually diagnosed by means of biopsies of pleura or peritoneum. Diagnosis of MM can be a challenge due to (i) the atypical features of mesothelial cells or lack of cells in the pleural or peritoneal fluids [8] and (ii) difficulties in differentiating MM from other afflictions, such as metastatic adenocarcinoma and benign pleural inflammation [4]. To resolve these problems, various approaches have been developed, including immunohistochemical assays [4,9]. Although immunohistochemical markers can be highly specific and sensitive, a substantial sample of tumor tissue is necessary for accurate diagnosis, requiring invasive surgery [10]. Therefore, the development of MM-specific molecular markers that could allow a diagnosis through the analysis of blood, pleural fluid or other bodily fluids and that does not require the presence of intact cells, would be highly desirable.

The first phase of marker development is the discovery of candidate genes or proteins by identifying molecular changes specific for tumor presence [11]. Exciting progress has been made in developing protein-based as well as expression-based (mRNA-derived) molecular markers for MM [1,12—18]. While these approaches are valuable, development of DNA-based markers would provide a powerful complementary approach with the advantages that the signal is exponentially amplifiable and relatively stable in bodily fluids. DNA methylation consists of the addition of a methyl group to the 5-position of cytosine in the context of a CpG dinucleotide. Hypermethylation of clusters of CpG dinucleotides (''CpG islands'') in the promoter regions of genes appears to lead to transcriptional silencing, and is thought to be a very common mechanism for the inactivation of tumor suppressor and growth regulatory genes in cancer [19,20]. Different types of cancer show distinct DNA methylation profiles, suggesting that it should be possible to develop cancer-type specific methylation signatures [21]. The power of DNA methylation as a marker derives not only from its ability to be detected in a wide variety of samples (from fresh specimens to bodily fluids and archival paraffin-embedded specimens) but also from the defined localization of the lesion (in promoter CpG islands of genes), allowing the design of gene-specific, targeted probes [22]. To date, findings regarding the role of DNA methylation in MM are still rather limited [23—35].

The goals of this study were dual: to identify new MM-specific methylation markers that could be used to develop highly specific and sensitive panels to effectively diagnose MM, and to explore possible relationships between methylation profiles and clinicopathological patient characteristics. Associations with patient features could be of importance for treatment or prognostication, and could also provide insights into the molecular mechanism of MM development. We examined the methylation status of 28 CpG islands of genes encoding tumor suppressor proteins and other growth regulatory proteins in 52 MM samples. For comparison, we analyzed 38 non-tumor lung samples from patients with lung carcinomas. The choice of loci was based on previous exploratory studies in our laboratory ([24] and unpublished data) as well as reports in the literature. Our data show that CpG island hypermethylation is common in MM, and that analysis of methylation profiles can provide MM-specific methylation markers, as well as insights into the potential role of epigenetic changes in the development and progression of MM.

2. Materials and methods

2.1. Study subjects and tissue samples

MM samples were obtained from 52 patients treated by one of us (HIP) at the Karamanos Cancer Institute between May 2000 and February 2004. Study subjects included 42 males and 9 females (gender unknown for one sample) ranging from 44 to 83 years old at time of surgery (median: 64 years old, age unknown for one subject). Thirty-nine of the 49 subjects were exposed to asbestos (asbestos exposure data was incomplete for three subjects). Histological subtypes were epithelioid (35), mixed (9) and sarcomatoid (4), with incom-