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Tumour Review

The established and future biomarkers of malignant pleural mesothelioma

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ABSTRACT

Malignant pleural mesothelioma (MPM) is an asbestos-related cancer with a median survival of 12 months. The MPM incidence is 1–6/100,000 and is increasing as a result of historic asbestos exposure in industrialized countries and continued use of asbestos in developing countries. Lack of accurate biomarkers makes diagnosis, prognostication and treatment prediction of MPM challenging. The aim of this review is to identify the front line of MPM biomarkers with current or potential clinical impact. Literature search using the PubMed and PLoS One databases, the related-articles function of PubMed and the reference lists of associated publications until April 26th 2015 revealed a plethora of candidate biomarkers. The current gold standard of MPM diagnosis is a combination of two positive and two negative immunohistochemical markers in the epithelioid and biphasic type, but sarcomatous type do not have specific markers, making diagnosis more difficult. Mesothelin in serum and pleural fluid may serve as adjuvant diagnostic with high specificity but low sensitivity. Circulating proteomic and microRNA signatures, fibulin-3, tumor cell gene-ratio test, transcriptomic, lncRNA, glycopeptides, pleural fluid FISH assay, hyaluronate/N-ERC mesothelin and deformability cytometry may be important future markers. Putative predictive markers for pemetrexed–platinum are tumor TS and TYMS, for vinorelbine the ERCC1, beta-tubuline class III and BRCA1. Mutations of the BAP1 gene are potential markers of MPM susceptibility. In conclusion, the current status of MPM biomarkers is not satisfactory but encouraging as more sensitive and specific non-invasive markers are emerging. However, prospective validation is needed before clinical application.

Introduction

Malignant mesothelioma (MM) is a highly lethal tumor of the pleura and peritoneum (common ratio 4:1) and rarely pericardium and tunica vaginalis testis [1,2]. Inhalation of asbestos is the carcinogenic factor in more than 80% of cases [3], but exposure to erionite, alpha-emitting contrast medium or irradiation of the thorax or abdomen in young age are also verified risk factors [1]. Initially, only occupational asbestos exposure was considered dangerous, but subsequent research has shown that environmental exposure by sharing residence with an asbestos worker and even living near an asbestos-emitting location increases risk considerably [1,3].

The diagnosis of malignant pleural mesothelioma (MPM) can be challenging due to similarities in clinical presentation and histological appearance of MPM, primary lung carcinoma, pleural metastases, reactive pleural diseases and rare pleural malignancies [4]. International pathological classification distinguishes three histopathological subtypes; epithelioid, sarcomatous, and biphasic MPM. The epithelioid and biphasic subtypes, which comprise 75–95% of all cases, have a relatively well-characterized immunophenotype [4]. On the other hand, relatively few studies of sarcomatous MPM have been reported and the histopathological diagnosis of sarcomatous MPM is still challenging [4,5].

MPM is a relatively chemo- and radio-resistant malignancy, and patients treated with pemetrexed–cisplatin have a median survival

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of 12.1 months, but occasionally long-term survivors are seen [6]. Radiation monotherapy has been disappointing, but encouraging results were shown with postoperative Intensity Modulated Radiation Therapy (IMRT) [7]. However, the only study were patients were randomized to trimodal treatment of chemotherapy, extra-pleural pneumonectomy (EPP) and radiotherapy by IMRT versus chemotherapy alone (MARS study), did not show any difference in survival [8]. The MARS study had, though, considerable limitations, as 44.6% of the patients did not proceed to randomization because of disease progression, patient choice or inoperability. Furthermore, only 66.7% of the patients randomized to EPP completed surgery, and 23.1% of the non-EPP patients decided to have EPP or other surgery off trial. Currently, radical pleurectomy/decortication of pleura has been proposed as a less traumatic procedure with decreased morbidity and encouraging data showed that combined with chemotherapy and high-dose radiation therapy the overall survival was 33 months in a small cohort [9,10]. Symptomatic treatment of recurrent pleural fluid with talc pleurodesis decreasing pleural fluid, improve respiration, and has even shown to increase survival in small series [11]. Psychological backing of patient and family and good palliative care is of utmost importance.

Early diagnosis of MPM could increase the overall survival, but pre-clinical biomarkers are not yet available [12]. Prognosis is positively correlated with epithelioid subtype, low stage, performance status, female gender and young age, but biomarkers of response and outcome are still not in clinical use [13]. The aim of this review is to present the current and the most promising future MPM biomarkers.

Methods

The PubMed and PLoS One databases and the reference lists of associated publications were used in this literature search with the following keywords: "malignant mesothelioma" combined with "biomarkers", "immunohistochemistry", "BAP-1", "deformability cytometry", "fibulin-3", "genome profile", "hyaluronan", "long non-coding RNA", "mesothelin", "microRNA", "osteopontin", "proteomics" and "soluble mesothelin related protein". No lower data limit was applied and only articles written in English were reviewed. All literature published until April 26th 2015 was included with no chronological limit.

Results and discussion

The search revealed a plethora of MPM biomarkers described with varying specificity and sensitivity. Markers in tumor and in body fluids with a current or promising clinical impact are discussed in this review.

Diagnosis by immunohistochemistry

Histopathological analysis of MPM tumor samples including immunohistochemistry (IHC) is the gold standard of diagnosis. Nevertheless, the most recent International Mesothelioma Interest Group (IMIG) guidelines suggest that the cytopathological diagnosis of epithelioid and biphasic MM, when supported by ancillary techniques (mainly immunocytochemistry and/or molecular biology, electron microscopy, biomarker analyses) is also a reliable technique [14]. It presents with equal positive predictive value but somewhat lower sensitivity in comparison with histopathology [14]. Thus, despite its less invasive nature it is regarded as a complement to the tumor biopsy. The International Mesothelioma Interest Group (IMIG) recommendations on MM IHC diagnosis require a panel of at least two immunoreactive and two non-immunoreactive markers to set the MM diagnosis [4]. The IHC analyses differentiate between epithelioid and biphasic MPM versus metastatic/directly infiltrating carcinoma/benign mesothelial proliferation, as well as between sarcomatous MPM versus primary/directly infiltrating/metastatic sarcomas/benign mesothelial proliferation [4]. The diagnosis of sarcomatous MPM is the most difficult due to lack of specific markers. In sake of brevity we will focus on the most common differential diagnostic situation in the clinic, the epithelioid MPM versus lung and breast adenocarcinomas. Highly conflicting results from different laboratories are often recorded in the literature, arising from different types of tumor samples submitted for immunostaining, tissue fixation and processing issues, varying sensitivity and specificity of the primary antibodies and protocols used, different stainer platforms and varying interpretation of immunostaining results [4,15,16]. However, according to the current studies and consensus reports, the most important markers in the differential diagnosis of MPM and lung and breast adenocarcinoma are the "mesothelioma markers" calretinin (CR), cytokeratin 5 (CK5), podoplanin (PDP) and Wilms' tumor-1 protein (WT1), the "broad spectrum adenocarcinoma markers" carcinoembryonic antigen (CEA), claudin-4 (CL4), and epithelial cell adhesion molecule (EpCAM), the "lung adenocarcinoma marker" thyroid transcription factor-1 (TTF1), and the "breast adenocarcinoma markers" estrogen receptor alpha (ER) and mammaglobin (MG) (Table 1). Selected mesothelioma markers are described in more detail below. Fig. 1 shows typical MPM infiltration and staining reactions of frequently applied IHC markers.

Calretinin

Calretinin is a calcium-binding protein of the EF-hand family, abundantly expressed in neurons and believed to play a key-role in somatosensory transduction [17]. Of all the immunomarkers, calretinin seems to be the most valuable in differentiating MPM from lung and breast adenocarcinoma, provided that only widespread nuclear reaction is considered positive [18,19]. There are several commercially available and validated monoclonal antibodies for calretinin (e.g., DAK-Calret1, 5A5, CAL6, and SP65) and a rabbit polyclonal antibody (18-0211, Invitrogen/Zymed). Efficient high-pH heat induced epitope retrieval (HIER) in combination with a sensitive visualization system is mandatory for optimal performance [19]. Calretinin can mainly be used in the diagnosis of epithelioid MPM, as its expression is diminished in areas with sarcomatous differentiation, and it has limited value in discriminating MM from serous or squamous carcinomas [18,20].

Cytokeratin 5

Cytokeratins (CK) are intermediate filaments located in the cytoplasm of virtually all epithelial cells and subsets of nonepithelial cells including mesothelial cells [21]. More than 70 CK subtypes have been identified. The large majority of MMs but also squamous cell carcinoma, basal-like breast carcinoma, ovarian serous and endometrioid carcinoma are CK5 positive while lung and breast adenocarcinomas are mostly negative [16,22]. Among several antibodies the clone XM26 has shown the best performance [23]. In contrast clone D5/16B4 (which also detects CK6), and clone 34betaH11 may give false positive staining reactions. Efficient high-pH HIER in combination with a sensitive visualization system is mandatory for optimal performance.

Podoplanin

Podoplanin is a sialoglycoprotein primary detected in podocytes, involved in embryonic development, and expressed in several normal tissues, including lymphatic endothelial cells and mesothelium [5]. It is frequently expressed in MPM, seminoma and angiosarcoma but less often in breast adenocarcinomas and