Human Pathology (2016) 55, 72–82

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Genomic copy number alterations in 33 malignant peritoneal mesothelioma analyzed by comparative genomic hybridization array☆,☆☆

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Received 19 February 2016; revised 1 April 2016; accepted 16 April 2016

Keywords:
Comparative genomic hybridization;
Copy number alterations;
Malignant peritoneal mesothelioma;
Asbestos;
VEGF-B

Summary Malignant peritoneal mesotheliomas (MPM) are rare, accounting for approximately 8% of cases of mesothelioma in France. We performed comparative genomic hybridization (CGH) on frozen MPM samples using the Agilent Human Genome CGH 180 K array. Samples were taken from a total of 33 French patients, comprising 20 men and 13 women with a mean (range) age of 58.4 (17-76) years. Asbestos exposure was reported in 8 patients (24.2%). Median (range) overall survival (OS) was 39 (0-119) months. CGH analysis demonstrated the presence of chromosomal instability in patients with MPM, with a genomic pattern that was similar to that described for pleural mesothelioma, including the loss of chromosomal regions 3p21, 9p21, and 22q12. In addition, novel genomic copy number alterations were identified, including the

☆ Competing interests: The authors have declared conflicts of interest with Astra Zeneca, Novartis, Roche.

☆☆ Funding/Support: This work was supported by the Rhone-Alpes Auvergne region of France (Groupement Interrégionaux de Recherche Clinique et d'Innovation [GIRCI] and Plateforme d'Aide à la Recherche Clinique en Cancérologie – Rhones-Alpes Auvergne [PARCC-ARA]) and the Lyon Research Innovation for Cancer (LYRIC) (Grant no: INCa-DGOS-4664). RENAPE is supported through an annual grant of the LFSS (Loi de Financement de la Sécurité Sociale) under MIGAC (Mission of General Interest and Contract support).

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http://dx.doi.org/10.1016/j.humpath.2016.04.015

15q26.2 region and the 8p11.22 region. Median OS was associated with a low peritoneal cancer index ($P = .011$), epithelioid subtype ($P = .038$), and a low number of genomic aberrations ($P = .015$), all of which constitute good prognostic factors for MPM. Our results provide new insights into the genetic and genomic background of MPM. Although pleural and peritoneal mesotheliomas have different risk factors, different therapeutics, and different prognosis; these data provide support to combine pleural and peritoneal mesothelioma in same clinical assays.

1. Introduction

Mesotheliomas are malignant tumors of the serous mesothelial tissue lining several body cavities, including the pleura, peritoneum, pericardium, and testes [1,2]. The pleura is the most common site of diagnosis and accounts for the majority of mesotheliomas, while approximately 30% of cases are peritoneal in origin [2]. Risk factors for mesothelioma include occupational or industrial exposure to asbestos fibers, genetic predisposition, and exposure to radiation [3]. The different types of mesothelioma are generally considered to be distinct pathologies, each with their own particular risk factors, therapeutic strategies, and prognosis. For example, whereas pleural mesothelioma is strongly associated with asbestos exposure, this relationship has not been clearly demonstrated for peritoneal mesothelioma [4].

With regard to histologic classification, there are 3 subtypes of mesothelioma: epithelioid, sarcomatoid, and biphasic (mixed epithelioid/sarcomatoid). Epithelioid mesothelioma is by far the most common histological subtype, accounting for 50% to 70% of cases [2]. Epithelioid peritoneal mesotheliomas treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) have a relatively good prognosis, with 5-year survival rates of 40% to 70% [5]. By contrast, sarcomatoid mesotheliomas are the most aggressive of the three histological subtypes and typically have a poor prognosis [6].

Studies of the genetics and genomics of mesotheliomas have aimed to gain insights into the molecular etiology of this disease. Homozygous deletion of the 9p21 region, containing the key cell cycle regulators cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and *ARF*, was first reported to be a common molecular event in mesothelioma cells by Illei et al in 2003 [7]. The *BRCA-associated protein 1* (*BAP1*) gene at 3p21 is another gene that is frequently lost or inactivated in mesothelioma [8]. In addition, germline mutations in *BAP1* have been shown to predispose carriers to mesothelioma [9].

Genomic copy number alterations (CNAs) involving deletions at 1p, 3p, 6q, 9p, and 22q are frequently observed in pleural mesothelioma [8,10–15]. Serio et al [16] performed a comparative genomic hybridization (CGH) analysis of two peritoneal mesothelioma samples arising primarily in the hernial sac. These authors reported that copy number gains were more frequent than losses and that the common deletions at 1p, 3p, 6q, 9p, and 22q identified in pleural mesothelioma were not found in these peritoneal samples [16]. More recently, Alakus et al [17] performed CGH analysis of nine peritoneal mesothelioma samples and identified a deletion of the 3p21 locus, containing the *BAP1* gene, in three samples (33.3%). Deletions of *CDKN2A* [18], *BAP1* [19], and the tumor suppressor *NF2* [20] were all identified by fluorescent in situ hybridization data from peritoneal mesothelioma samples. Overall, these results are suggestive of a similar pattern of genomic alterations in pleural and peritoneal mesotheliomas; however, additional data are needed to further characterize the molecular genomic events associated with peritoneal mesothelioma.

Here we describe the use of array-based CGH to characterize the CNA profile in a set of peritoneal mesothelioma samples. In addition, we assessed the prognostic values of clinicopathological characteristics and CNAs on overall survival (OS) in patients with peritoneal mesothelioma.

2. Material and methods

2.1. Patients and samples

Patients were treated with cytoreductive surgery and HIPEC as described previously [21]. Tumor and normal tissue samples were obtained during surgery with informed consent obtained from patients included in the Réseau National de Prise en Charge des Tumeurs Rares du Péritoine (RENAPE) network [22]. Diagnosis was confirmed based on surgical, histological and immunohistochemical characteristics (Fig. 1) in line with consensus guidelines established by the International Mesothelioma Interest Group [23] and the Mésothéliomes Malins Pleuraux et Tumeurs Péritonéales Rares network [24]. Calretinin immunohistochemistry was performed with a polyclonal antibody from Zytomed, Berlin, Germany, at the dilution 1:100. The peritoneal cancer index (PCI) score and the completeness of cytoreduction (CC) score were used to characterize the extent of the disease intra-operatively [25,26]. A CC score of 0 (CC-0) indicated that no macroscopic tumor nodules remained; CC-1 indicated that no nodules >2.5 mm in diameter remained; CC-2 indicated that nodules between 2.5 mm and 2.5 cm in diameter remained; CC-3 score indicated that nodules >2.5 cm remained.

The peritoneal mesothelioma tumor collection comprised 35 frozen samples obtained between 1998 and 2014 from the