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Immune biomarkers PD-1/PD-L1 and TLR3 in malignant pleural mesotheliomas

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Summary Malignant pleural mesothelioma (MPM) is an aggressive tumor with no effective therapy. However PD-L1/PD-1 immunity checkpoint therapies gave encouraging results; TLR3 is a programmed death factor, which triggering up-regulates PD-L1. As PD-1/PD-L1 blocking antibodies could restore antitumor immune responses alone or in combination with TLR3 agonists, we investigated PD-L1/PD-1 and TLR3 expressions in MPM to select patients for immunotherapy. Sixty-eight pleural surgical specimens, including 58 MPM (epithelioid, n = 34; biphasic, n = 11; sarcomatoid, n = 13) and 10 benign lesions, were studied. PD-L1 expression was assessed using E1L3N and SP142 clones in tumor cells (TCs) and in tumor-infiltrating lymphocytes (TILs) (positivity threshold of 1%), and compared with overall survival. PD-1, CD3 and CD8 expression by TILs, and TLR3 expression by TCs were analyzed

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concomitantly. PD-L1 was more expressed by sarcomatoid subtype than by other MPM (62% versus 23% and 9% for E1L3N; 38% versus 11% for SP142) (P = .01 and .04, respectively). Specificity and sensitivity of E1L3N and SP142 were of 53% and 98%, and 90% and 86%, respectively. PD-L1 expression by TILs and TCs correlated for SP142 (P = .023), and PD-L1 SP142 expression by TCs was associated with shorter overall survival (P = .016). TLR3 was expressed in most MPM, but weakly in sarcomatoid MPM. We confirm by comparing two commercially available antibodies that PD-L1 expression is higher in sarcomatoid MPM and correlates with a shorter survival. Whereas TLR3 agonists could be tested in MPM expressing TLR3, the sarcomatoid subtype could benefit from anti–PD-L1/PD-1 therapies alone or in combination.

1. Introduction

Malignant pleural mesothelioma (MPM) is a rare aggressive tumor, mainly related to a prolonged exposure to asbestos [1]. It concerns predominantly patients older than 60 years and, very rarely, young people. However, despite the suppression of asbestos, MPM incidence decreases slower than expected and even tends to increase in women [2]. MPM treatment is classically based on combination of pemetrexed and platinum but its prognosis remains poor, with a median survival time estimated at 12 months [3]. The development of novel therapeutic strategies based on translational research is thus highly anticipated [4].

Histologically, MPMs are divided in epithelioid, biphasic and sarcomatoid subtypes according to the World Health Organization classification of pleural tumors [5], the epithelioid MPM being the most frequent with the best prognosis, whereas the sarcomatoid MPM is the rarest type but with the poorest prognosis. Tumor microenvironment plays a major role in tumor progression, favoring tumor cell evasion from adaptive immunity and T-cell checkpoint pathways [6]. Among factors involved in immune response inhibition by the tumor cells, PD-1 (Programmed Death-1) and its main ligand PD-L1, also known as B7-H1 or CD274, have been studied in many tumors and several anti PD-1 or PD-L1 strategies under development have given very encouraging results [7,8].

PD-1, a receptor that belongs to the CD28 family, is expressed by T cells, B cells, monocytes, natural killer cells, and many tumor-infiltrating lymphocytes (TILs) [9]. PD-L1 is one of the main ligands of PD-1 and participates in T-cell co-stimulation, functioning as a negative regulator of immunity. PD-L1 is expressed by normal resting T cells, B cells, dendritic cells, macrophages, endothelial cells, and pancreatic islet cells [9]. In addition, PD-L1 is highly expressed by most carcinomas including those of the lung, thymus, breast, liver, colon, and bladder, but minimally expressed by adjacent normal tissue, with a role in attenuating antitumor immune response [10]. Cancer cells expressing PD-L1 have been shown to increase apoptosis of antigen-specific human T-cell clones [11] and to inhibit CD4+ and CD8+ T-cell activation in vitro. Furthermore, mice succumb rapidly to tumors transfected with PD-L1 and when PD-1/PD-L1 interaction is blocked, in vivo tumorigenesis is significantly inhibited [12]. These properties have been used for the development of checkpoint inhibition therapeutic strategies in many clinical trials [7,8,13].

Toll-like receptor 3 (TLR3) belongs to the Toll-like receptors family, comprising 10 members in humans. It is a highly glycosylated type I membrane receptor involved in viral double-strand RNA recognition and initiation of immune response [14,15]. TLR3 is also a death factor, triggering the release of apoptotic bodies [14,16] inducing cancer cell apoptosis. In addition, TLRs regulate cancer immunity and tolerance through innate immune responses mediated by regulatory T cells, dendritic and other immune cells [17,18]. TLR3 can stimulate cancer cells to secrete pro-inflammatory cytokines and chemokines involved in anticancer immune responses [14]. It is expressed by normal immune, epithelial and endothelial cells, and by breast [19], prostate [20], and head and neck carcinomas [21]. TLR3 triggering induces a strong up-regulation of both MHC class I and PD-L1 on neuroblastoma cells, which suggests a combination of synthetic TLR ligands with PD-L1 blocking agents to restore anti-tumor immune responses [22]. Our aim was thus to evaluate PD-L1 and TLR3 expressions in various histological subtypes of MPMs, in order to select MPM patients eligible for immunotherapies.

2. Materials and methods

Sixty-eight surgical FFPE (formalin-fixed and paraffin-embedded) pleural specimens were retrieved from the Grenoble University Hospital files between 1993 and 2014. They comprised 58 MPMs, including 34 epithelioid (EMM), 13 sarcomatoid (SMM) and 11 biphasic (BMM) subtypes according to the 2015 World Health Organization classification of pleural tumors [5], and 10 benign lesions of the pleura, including 6 sub-acute to chronic pleuritis and 4 mesothelial hyperplasia with or without pleural fibrosis or plaques. MPM diagnoses were confirmed by the national referent center National Referent Center Mesopath-Pr F Galateau-Sallé in Caen. Patients' clinical data are listed in Table 1. All patients presented with advanced clinical stages III or IV according to the pTNM staging system for mesothelioma.