Human Pathology (2018) 72, 135–143

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Mismatch repair–deficient colorectal cancer: a model of immunogenic and immune cell–rich tumor despite nonsignificant programmed cell death ligand-1 expression in tumor cells☆

Glen Le Flahec MD[a], Bogdan Badic MD[b], Briac Guibourg MD[a], Laurent Doucet MD[a], Jean-Pierre Bail MD, PhD[b,c], Pascale Marcorelles MD, PhD[a,c], Ulrike Schick MD, PhD[c,d], Arnaud Uguen MD, PhD[a,c,e,*]

[a]*CHRU Brest, Service d'anatomie et cytologie pathologiques, Brest, F-29220, France*
[b]*CHRU Brest, Service de chirurgie viscérale, Brest, F-29220, France*
[c]*Université Européenne de Bretagne, Brest, F-29220, France*
[d]*CHRU Brest, Service de Radiothérapie, Brest, F-29220, France*
[e]*Inserm, U1078, Brest, F-29220, France*

Received 6 July 2017; revised 12 September 2017; accepted 21 September 2017

Keywords:
Colorectal cancer;
Mismatch repair deficiency;
PD-1;
PD-L1;
Tumor immune microenvironment

Summary Mismatch repair–deficient (dMMR) colorectal cancers (CRCs) are good responders to anti–programmed cell death ligand-1 (PD-L1) immunotherapy, but the value of PD-L1 testing remains unclear. We studied PD-L1 expression and the tumor immune microenvironment in dMMR CRC as a model of good responders to immunotherapy. We examined 35 dMMR and 34 mismatch repair–proficient (pMMR) CRCs using immune cell markers (CD3, CD4, CD8, CD20, CD68, and FOXP3) as well as programmed cell death receptor-1 (PD-1) and PD-L1 immunohistochemistry staining in whole tumor specimens and tissue microarray slides to compare 4 PD-L1 immunohistochemistry clones (SP142, E1L3N, 22C3, and 28.8). We observed no significant difference in PD-L1 expression between dMMR and pMMR CRCs. Only 2 dMMR tumors had membranous PD-L1 staining. Expression of PD-L1 was greater in stromal immune cells of dMMR CRC, which also contained more numerous intraepithelial ($CD3^+$, CD8+, $FOXP3^+$, and $PD\text{-}1^+$) and stromal ($CD8^+$, $PD\text{-}1^+$) lymphocytes than did pMMR tumors. Immune cell quantification discriminated better between dMMR and pMMR tumors than did PD-L1 expression. Tumor heterogeneity and variations in PD-L1 expression were noted with different antibodies, especially for $PD\text{-}L1^+$ immune cells, which were more numerous at the invasion margin. Given the poor correlation with mismatch repair status and technical limitations, the value of PD-L1 testing to accompany the development of anti–PD-1/PD-L1 immunotherapy remains unclear. Further clinical trials are required to determine which parameters are valuable predictive biomarkers of the response to immunotherapy among mismatch repair status, PD-L1 expression, and immune cell quantification in CRC.

☆ Disclosures: The authors declare no source of support for this work and that they have no conflicts of interest related to this article.

* Corresponding author at: Department of Pathology, University Hospital Morvan, 2, avenue Foch, 29609 Brest, France.
E-mail addresses: arnaud.uguen@chu-brest.fr, arnaud.uguen@gmail.com (A. Uguen).

https://doi.org/10.1016/j.humpath.2017.09.019
0046-8177/

1. Introduction

Colorectal cancers (CRCs), most of which are adenocarcinomas, are the third most frequent cancer and the fourth most common cause of cancer-related death in the world [1]. The treatment for patients with CRC has been improved by a better understanding of the molecular mechanisms involved in tumor initiation and progression. For example, anti–epidermal growth factor receptor therapies are now approved by regulatory authorities to inhibit the mitogen-activated protein kinase pathway and tumor progression in patients with CRC lacking *KRAS-NRAS*–activating mutations [2]. Besides therapies targeting the oncogenic molecular mechanisms in the cancer cells themselves, some treatment strategies have allowed decreases in tumor progression by modifying the tumor stroma. For example, anti–vascular endothelial growth factor therapies impair tumor growth by acting on the tumor microvascular environment [3]. Recently, analysis of the interactions between cancer cells and the tumor immune environment has attracted attention by implying a potential therapeutic relevance for immunotherapy [4].

Survival and progression of cancer require that the immune system fail to recognize and eliminate the lesion. This failure involves many immune checkpoints that normally cause tumor-induced immunosuppression. The programmed cell death receptor-1 (PD-1)/programmed cell death ligand-1 (PD-L1) axis is one of these checkpoints [5,6]. PD-1 is a membranous receptor that is expressed by T and B lymphocytes and natural killer cells, natural killer T cells, and dendritic cells. By interacting with its ligands, particularly PD-L1, PD-1 down-regulates the activity of immune cells. PD-L1 is expressed physiologically by many kinds of cells, including dendritic cells, macrophages, and T and B lymphocytes. Its expression is induced by interferon-γ. Programmed cell death ligand-2 is another ligand of PD-1, which is expressed by macrophages, dendritic cells, and lymphocytes whose PD-1–related immunosuppressive effects seem inferior to those obtained by interaction with PD-L1 [5-8]. PD-L1 can be expressed by tumor cells either constitutively or after induction by the microenvironment, leading to immune tolerance and tumor progression [9].

Many immunotherapies targeting the PD-1/PD-L1 axis have been developed as anticancer drugs with sometimes impressive and encouraging results. A few drugs are approved for treatment of some cancers (eg, melanomas and non–small cell lung cancer), causing long-term tumor regression and improvement of global and disease-free survival in some patients whose tumors may overexpress PD-L1 [10]. This research has led to extensive (and heterogeneous from a technical point of view) screening for PD-L1 expression in many cancer types using immunohistochemistry (IHC) staining [11]. Nevertheless, to predict the response of a patient to these expensive treatments remains challenging because, to date, no biomarker, including PD-L1 expression in tumors, has proved to be sensitive and specific enough to predict clearly whether a given patient will be a good or a poor responder. Indeed, a "PD-L1^{-}" patient can respond to PD-1/PD-L1 blockade, and a "PD-L1^{+}" one can show no response. Besides PD-L1 expression, other parameters need to be studied to predict more accurately which patients will benefit from anti–PD-1/PD-L1 immunotherapy [12].

Le et al [4] demonstrated that a subset of CRCs consisting of mismatch repair–deficient (dMMR) cancers often respond to PD-1/PD-L1 blockade, whereas mismatch repair–proficient (pMMR) cancers do not. In their study, the importance of PD-L1 IHC was not obvious as a predictor of the response to anti–PD-1 pembrolizumab.

In this study, we focused on the immune environment and PD-L1 expression of dMMR CRC as a model of potential good responders to anti–PD-1 immunotherapy. We also compared several PD-L1 IHC protocols in CRC and considered tumor heterogeneity for PD-L1 expression in a set of dMMR and pMMR CRCs.

2. Materials and methods

2.1. Case selection and tumor sample processing

The cases included in this study were diagnosed at the Brest University Hospital between 2009 and 2015. We selected 2 sets of tumors according to their dMMR or pMMR status, as determined by MLH1, MSH2, MSH6, and PMS2 IHC staining and fragment-length polymorphism analysis of 5 microsatellites: BAT26, BAT25, NR21, NR22, and NR24. A sample was classified as unstable if at least 3 markers were unstable and IHC staining pointed to loss of proteins implicated in microsatellite instability. Both IHC staining and molecular analyses (including *KRAS*, *NRAS*, and *BRAF* mutations as well as *MLH1* promoter methylation status when appropriate) were conducted using formalin-fixed, paraffin-embedded tumor samples as part of the diagnostic workup for the therapeutic management of patients with CRC according to French National Cancer Institute guidelines.

The same formalin-fixed, paraffin-embedded tumor blocks were used, first, for whole slide–based analyses and, second, for tissue microarray (TMA)–based ones. The TMA blocks were constructed using the 3DHistech TMA Grand Master automated tissue microarrayer (3DHistech, Budapest, Hungary) with 7 spots (2-mm core diameter) per tumor sample. The present study was conducted according to our national and institutional guidelines and in compliance with the Declaration of Helsinki and after approval by our institutional review board (CHRU Brest, CPP no. DC-2008-214). All samples were included in a registered tumor tissue collection.

2.2. Immunohistochemistry

The CD3 (polyclonal antibody, 1:100; Dako, Glostrup, Denmark), CD4 (clone SP35, previously diluted; Roche Diagnostics, Meylan, France), CD8 (clone C8/144B, 1:25; Dako), CD20 (clone L26, 1:100; Dako), CD68 (clone PG-M1, 1:100; Dako), FOXP3 (clone FJK-16 s, 1:100; Thermo Fisher Scientific, Waltham, MA, USA), PD-1 (clone NAT105, previously diluted;