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Original article

PD-L1 expression in HNPCC-associated colorectal cancer

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ABSTRACT

Background: PD-L1 immunohistochemistry is predictive for molecular inhibitors of PD-1/PD-L1 immune checkpoint. Therefore, this study evaluated the PD-L1 expression in patients with Hereditary Non-Polyposis Colorectal Cancer (HNPCC).

Methods: Immunohistochemical expression of PD-L1 in carcinoma cells, stromal macrophages and lymphocytes of 40 HNPCC-patients with colorectal cancer was scored semi-quantitatively.

Results: Focal (2 cases) to extensive (2 cases) PD-L1-immunopositivity of carcinoma cells was detected in 4 out of 40 cases (10.0%). Stromal macrophages were immunopositive in 28 out of 40 cases (70.0%). Lymphocytes showed PD-L1 expression in 3 out of 40 cases (7.5%). Simultaneous immunopositivity of stromal macrophages and tumor cells was detected in two MLH1/PMS2-deficient and two MSH2/MSH6-deficient cases.

Conclusion: A subset of HNPCC-associated colorectal cancers in this study clearly showed PD-L1 expression of tumor epithelia and immune cells, therefore, the detection of PD-L1 status is useful.

1. Introduction

Programmed death ligand-1 (PD-L1) which acts by binding to PD-1 receptor can be expressed by both malignant cells and immune cells [1]. Tumoral PD-L1 expression can inhibit T cell activation suppressing antitumoral immune response [2]. Therefore, the inhibition of the PD-1/PD-L1 immune checkpoint is used as a promising therapeutic approach [3]. PD-L1 overexpression has been found in various malignant neoplasias like lung carcinoma [3,4], malignant melanoma [5], malignant lymphoma [6] and more.

In recent publications, the role of PD-L1 expression has been studied in MSI-deficient and −proficient colorectal cancers [2,7,8]. Though, an analysis of PD-L1 expression in exclusively Hereditary Non-Polyposis Colorectal Cancer (HNPCC)-associated colorectal cancers has not been done to our knowledge, yet. Nevertheless, Le and coworkers previously showed that PD-1 blockage of mismatch repair (MMR)-defective tumors results in good response rates [9].

Hereditary Non-Polyposis Colorectal Cancer, also referred to as Lynch syndrome, is the most common hereditary form of colorectal carcinomas and occurs as a result of MMR gene deficiency [10]. Germline defects in the MMR genes (MLH1, MSH2, MSH6, PMS2) have been identified to be linked to HNPCC, which is an autosomal dominant hereditary cancer syndrome [11]. Absence of these mismatch repair enzymes causes microsatellite instability which refers to randomly repeated short sequence patterns of DNA [12]. Microsatellite instability of HNPCC-associated cancers induces high mutational load provoking a strong antitumoral immune response [13]. Therefore, these patients benefit from Anti-PD-1/PD-L1-immunotherapy.

The aim of the present study was to determine immunohistochemical PD-L1 expression patterns in a well-characterized collective of 40 patients with HNPCC-associated colorectal cancers. Furthermore, it was of interest, whether MMR-associated mutational groups of HNPCC-patients show different patterns of PD-L1 expression.

2. Material and methods

2.1. Collective of tissue samples

In this study, 40 cases of HNPCC-associated colorectal cancer were analyzed. The paraffin blocks were taken from the archive of the German HNPCC Consortium comprising the years 2003–2014 as part of a larger study on susceptibility to hereditary nonpolyposis colorectal cancer [14]. Clinicopathologic features from the analysis of all colorectal cancer cases are summarized in Table 1.

17 cases showed immunohistochemical loss of MLH1-/PMS2- MMR enzymes, 21 cases showed loss of MSH2-/MSH6-MMR enzymes, 1 case was found to be solely MSH6- deficient and 1 case showed PMS2-loss. Diagnosis of HNPCC-associated neoplasia was further validated by microsatellite analyses of all cases and BRAF V600-mutation analyses of MLH1-/PMS2-deficient cases. All cases of the collective showed high microsatellite-instability (MSI-H). The MLH1-/PMS2-negative cases showed wildtype sequence for BRAF V600 mutations.

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Table 1
Patient collective.

	MLH1-/PMS2-	MSH2-/MSH6-	MSH6-	PMS2-
Age (years)				
< 50	9	12	0	1
≥50	8	9	1	0
Age (mean)	47.4	50.2	50	42
Gender				
Female	6	10	0	1
Male	11	11	1	0
T stage				
pTx	13	9	0	0
pT0	0	0	0	0
pT1	0	1	0	0
pT2	0	1	0	0
pT3	4	8	1	1
pT4	0	2	0	0
N stage				
pNx	13	10	1	0
pN0	4	7	0	0
pN1	0	2	0	1
pN2	0	2	0	0
M stage				
Mx	17	20	1	0
M0	0	0	0	1
M1	0	1	0	0
Grade				
0	0	0	0	0
1	0	4	0	0
2	9	9	1	0
3	8	8	0	1

Clinical information about tumor size, lymphonodal or hematogenous metastasis as well as tumor grading of these samples were documented if available.

2.2. Immunohistochemistry and immunofluorescence

Specimens were stained with a mouse anti-human PD-L1-antibody on an automated staining system according to the manufacturers' instructions (pharmDx, clone 28-8, DAKO, Glostrup, Denmark). The analyzed cells were considered PD-L1 positive if they showed membranous staining. Cells containing cytoplasmic staining were excluded from analysis. Tumor epithelia and stromal cells like macrophages and lymphocytes were analyzed separately.

Membranous PDL-1 expression of tumor epithelia and immune cells were scored according to the Cologne scoring system consisting of 6 categories (0–5) [15] (Table 2). The samples were independently scored by 2 persons (N.S and N.F) who were blinded for the results. In order to determine PD-L1 expression of cytokeratin-20-immunopositive tumor cells a double-staining with PD-L1 and cytokeratin-20 (CK-20, 1:400, rabbit anti-human, Cell Marque, Rocklin, CA, USA) was carried out as described before [16].

Table 2
Cologne scoring system for PD-L1 positive cells.

Cologne score score	% of positive cells
0	< 1%
1	≥1%, < 5%
2	≥5%, < 10%
3	≥10%, < 25%
4	≥25%, < 50%
5	≥50%

3. Results

3.1. Diagnostic validation of HNPCC-associated colorectal cancers

17 out of 40 cases (42.5%) displayed immunohistochemical loss of MLH1/PMS2 mismatch repair enzymes, 21 out of 40 cases (52.5%) showed MSH2/MSH6 loss. In addition, one case (2.5%) was MSH6- and one case (2.5%) PMS2-deficient. All cases (100.0%) showed high microsatellite instability (MSI-H) in PCR analyses. In order to rule out sporadic colon cancer cases within the MLH1-/PMS2-deficient subset of patients BRAF V600-mutation analyses were performed in all MLH1-/PMS2-deficient cases. All these 17 carcinomas showed wild type sequence for BRAF V600 indicating HNPCC-associated colorectal cancer.

3.2. Semiquantitative analyses of PD-L1 expression

4 out of 40 HNPCC-associated colorectal cancers (10.0%) showed differential expression of PD-L1 in carcinoma cells. In the immunohistochemical stainings, tumoral PD-L1 immunopositivity was often focal. Extensive and strong tumoral PD-L1-expression (score 4) was detected in 2 cases (Fig. 1A, B): 1 case with MLH1-/PMS2-loss and 1 case with MSH2-/MSH6-deficiency. Circumscribed and weak PD-L1 immunopositivity (score 1) was detected in 2 other cases showing MLH1-/PMS2-loss and MSH2-/MSH6-deficiency, respectively. All other tested carcinomas did not show tumoral PD-L1 expression at all.

Corresponding to the observations made in tumor cells macrophages showed differential PD-L1 immunopositivity. In 17 cases (42.5%) extensive PD-L1 positivity (scores 4–5, Fig. 1D) was observed. 11 cases (27.5%) showed circumscribed PD-L1 expression (scores 1–3) and 12 cases (30.0%) did not show PD-L1 expression. There was no difference in PD-L1-immunopositivity of macrophages between mutational groups showing tumoral MLH1-/PMS2-loss and MSH2-/MSH6-loss. Two cases with singular MSH6- and singular PMS2-loss showed maximum scores of 5 in macrophages. Tumor cells were immunonegative in both cases (score 0).

In the 4 cases with focal to extensive tumoral PD-L1 expression, stromal macrophages also showed extensive and strong PD-L1 immunopositivity (Fig. 1C, Fig. 2).

Lymphocytic infiltrates displayed circumscribed and weak PD-L1 expression in 3 out of 40 cases bearing MLH1-/PMS2-deficiency. Tumor cells in these cases were immunonegative.

4. Discussion

PD-L1/PD-1 checkpoint inhibitors play an essential role in treatment of several malignancies such as lung cancer [3], malignant melanoma [5], malignant lymphoma [6,17] and, more recently, also colorectal carcinoma [2,8,18,19].

Various studies determined immunohistochemical expression patterns of PD-L1 in colorectal cancer analyzing MMR-proficient and MMR-deficient cases. Rates of cases classified as PD-L1-immunopositive varied between the studies. Among these, MMR-deficient cases showed PD-L1-immunopositivity in 29% [2], 21% [18], 12% [7,8] and 9% [19]. Though, in the aforementioned studies the analyzed MMR-deficient colon carcinomas often contained BRAF V600-mutated cases, which are known to be associated with sporadic origin of colon cancer but not with HNPCC [20]. To our knowledge, this the first study to analyze immunohistochemical PD-L1 expression in a well-defined collective of HNPCC-associated colorectal cancer cases. In our study, a collective of 40 HNPCC-associated colorectal cancers showed weak tumoral PD-L1 expression in 2 cases and strong expression in further 2 cases (altogether 10%). Therefore we conclude that a small subset of HNPCC-associated colorectal cancers show PD-L1 expression of tumor cells. The relatively high rate of MMR-deficient, PD-L1 immunopositive colon cancers in other studies might be induced by sporadic colon cancer cases with loss of MLH1-/PMS2-expression and BRAF V600-