Programmed death 1 (PD-1) lymphocytes and ligand (PD-L1) in colorectal cancer and their relationship to microsatellite instability status

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Abstract

Background: PD-1 activation by its ligands (PD-L1 and PD-L2) inhibits T-cell activation and plays a role in cancer progression. PD-L1 is widely expressed in many cell types in tumor microenvironment. In contrast, expression of PD-1 is restricted to a small subset of T-lymphocytes. Inhibition of PD-1/PD-L1 interaction showed no benefit in a small number of colorectal cancers (CRC) studied in clinical trials. We investigated tumor infiltrating PD-1+ lymphocytes and PD-L1 expressing cells in CRC to gain insight in their role as biomarkers.
Methods: 87 CRC cases (60 microsatellite stable and 27 with high microsatellite instability) were profiled (Caris Life Sciences) for the presence of PD-1 and PD-L1 expressing cells, mismatch repair proteins, DNA microsatellite instability (MSI) and select cancer genes sequences (NGS). Only intraepithelial PD-1+ lymphocytes (IEL) and aberrantly expressed PD-L1 on carcinoma cells were considered specific.
Results: PD-1+ IEL were detected in approximately 50% of CRCs. Microsatellite stable (MSS) cancers were frequently (61%) negative for PD-1. Microsatellite instability-high (MSI-H, both Lynch syndrome and sporadic) were significantly ($p<0.05$) more frequently infiltrated with PD-1+ IEL than MSS (77% MSI-H vs. 39% MSS). Similarly, PD-L1+ cancer cells were more common in MSI-H (38%) than in MSS (13%, $p<0.005$), but the expression was patchy in all cases. Concurrent PD1+ IEL and PD-L1 cancer cells were seen in 32% of MSI-H and 4% of MSS cancers ($p=0.008$).
Conclusions: Consideration of immune checkpoint therapies for colorectal cancer needs to consider the presence of PD-1 lymphocytes and cancer cell specific PD-L1 expression. PD-1+ IEL and PD-L1+ cancer cells are more frequent in MSI-H than in MSS colorectal cancers, which are rare in general CRC population.

Introduction

Programmed death-1 (PD-1, CD279) is the immune suppressive molecule that is upregulated on activated T cells and other immune cells. It is activated by binding to its ligand B7-H1 (PD-L1, CD274), which constitute "check-point molecules" (1). Aberrant PD-L1 expression had been observed on cancer cells, leading to the development of immune checkpoint cancer therapies ["next-generation immunotherapy"] which have shown promising results in recently published late phase clinical trials (2). Blockade of the PD-1 and PD-L1 interaction led to good clinical responses in several, but importantly not in all cancer types, and their complex cellular expression and interaction may underlie these selective responses.

Most of the published papers focused on prognostic relevance of PD-1/PD-L1 while little is known about their relationship to molecular genetic alterations in CRC (3).

In the present study, we analyzed distribution of PD-1+ tumor infiltrating lymphocytes (TIL) and aberrant cancer cells PD-L1 expression in 2most common CRC molecular subtypes: microsatellite stable (MSS) and microsatellite instable (MSI-H).

Materials and Methods

Tumor samples

The study cohort consisted of 87 tumor samples (both primary and metastatic) from 60 MSS and 27 MSI-H CRC. Classification of the tumors (MSS vs. MSI-H) was based on immunohistochemical expression

Immunohistochemistry

The presence of PD-1 lymphocytes was evaluated using monoclonal antibody NAT105 (Cell Marque) while the expression of PD-L1 was analyzed using human B7-H1 antibody (R&D Systems). IHC assays were performed using automated staining techniques (Benchmark XT, Ventana, USA and AutostainerLink 48, DAKO, Denmark).
Due to the biopsy size-related dependence on the number of PD-1 lymphocytes we evaluated their density using a hot-spot approach, analogous to the previously described method for measuring neoangiogenesis (4). The whole tumor sample was reviewed at a low power (4x objective) and area of highest density of tumor infiltrating lymphocytes in direct contact with malignant cells of the tumor at 400x visual field (40x objective x 10x ocular) was recorded (number of PD-1+ TIL/HPF). The intensity of the cancer cells expression of PD-L1 was recorded on a semiquantitative scale (0-3+): 0 for no staining, 1+ for weak cytoplasmic staining, 2+ moderate membranous and cytoplasmic staining and 3+ strong membranous and cytoplasmic staining. Percent of tumor cells expressing PD-L1 at the highest intensity was recorded.

Results

PD-1 and PD-L1 expression in normal tissues

PD-1+ lymphocytes were identified in reactive, peri-tumoral lymphoid follicles, which served as an internal positive control while PD-L1 was expressed in the tumor microenvironment and was used as internal positive control.

Association of PD-1 and PD-L1 expression with genotypic characteristics of the CRC

In the colon cancer cohort, MSI-H tumors exhibited significantly higher rate of positivity for PD-1+ TILs then microsatellite stable (MSS) colon cancers (78% vs. 39%, $p=0.002$, Fisher's exact test) (Table_1). Also, the proportion of PD-L1+ cancers was significantly higher in MSI-H than in the MSS colon cancers (38% vs. 13%, $p=0.02$, Fisher's exact test) (Table 1, Figures 1 and 2).

Results, *continued*

Of note, MSI-H cases were predominantly stage I and II (75%) whereas the majority of the MSS cases were at advanced stage (III and IV, 93%) ($p<0.001$). Both PD-1 and PD-L1 positivity significantly decreased with the tumor stage ($p=0.021$ and 0.031, respectively).

Colon cancer subtypes (n=87)	PD-1 expression (TILs) (% and range)	PD-L1 (tumor cells) (%)	Concurrent PD-1/ PD-L1 expression (%)
MSS colon cancers (n=60)	39% (1-11)	13%	4%
MSI-H colon cancers (n=27)	77% (1->20)*	38%*	32%*

*Significantly higher ($p<0.05$)

Table 1. Both PD-1 and PD-L1 exhibited significantly higher expression in MSI-H colon cancers in comparison with MSS cases.

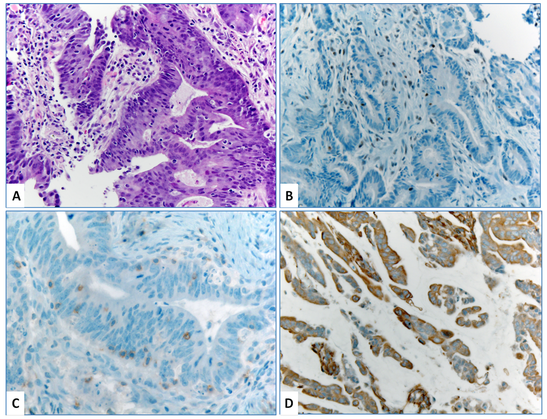

Figure 1A-D. Hematoxylin and Eosin stained section (A) of a case of colorectal adenocarcinoma with microsatellite instability caused by the loss of MSH2 protein (B); note the presence of abundant intra-epithelial PD-1+ lymphocytes (C) and the aberrant PD-L1 expression in cancer cells (D).

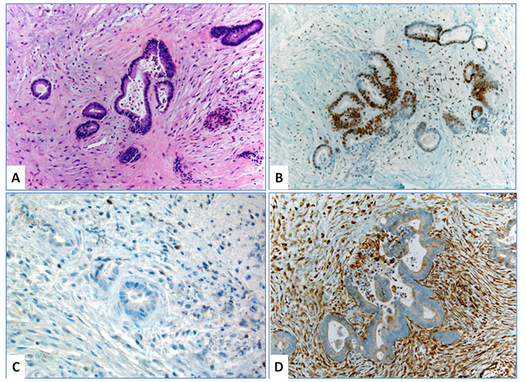

Figure 2A-D. Hematoxylin and Eosin section (A) of a case of MSS colon adenocarcinoma; Note the retained MSH2 protein expression (B); PD-1 stain reveals rare positive lymphocytes in the tumor stroma, but not within cancer epithelium (C); PD-L1 expression is present in tumor microenvironement, but not in the cancer epithelium (D).

Conclusions

- Consideration of immune checkpoint therapies for colorectal cancer needs to consider the presence/location of PD-1 lymphocytes and cancer cell specific PD-L1 expression, to refine their value as a potential companion diagnostics.
- MSI-H CRC is more likely to have PD-1+ TILs and to express PD-L1, than the MSS CRC.
- The aberrant cancer cells' expression of PD-L1 may be affected by the stage.

References

1. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res 2013;19:1021-34.
2. Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy--inhibiting programmed death-ligand 1 and programmed death-1. Clin Cancer Res 2012;18:6580-7.
3. Droeser RA, Hirt C, Viehl CT, et al. Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer 2013;49:2233-42
4. Weidner N. Measuring intratumoral microvessel density. Methods Enzymol 2008;444:305-23.