IN THE SPOTLIGHT

The Microsatellite Instable Subset of Colorectal Cancer Is a Particularly Good Candidate for Checkpoint Blockade Immunotherapy

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Summary: The microsatellite instable (MSI) subset of colorectal cancer exhibits an active Th1/CTL immune microenvironment, probably due to recognition of a high number of tumor neoantigens. However, the high expression of checkpoint molecules PD-1, PD-L1, CTLA-4, LAG-3, and IDO in MSI colorectal cancer distinguishes MSI from microsatellite stable colorectal cancer and creates an immunosuppressive microenvironment that may help MSI tumors evade immune destruction by the infiltrating immune cells. Though colorectal cancer does not have a good response rate to PD-1 pathway immunotherapy, these results suggest that the MSI subset of colorectal cancer is a particularly good candidate for checkpoint immunotherapy. *Cancer Discov; 5(1); 16–8. ©2015 AACR.*

See related article by Llosa et al., p. 43 (3).

Microsatellite instable (MSI) colorectal cancer comprises approximately 15% of sporadic colorectal cancer and most familial colorectal cancer, whereas microsatellite stable (MSS) colorectal cancer comprises the remainder (1). MSI is typically diagnosed by the variable length of DNA microsatellites (mononucleotide and dinucleotide repeats; ref. 1). This variation is a consequence of epigenetic silencing or mutation of DNA mismatch repair genes (1, 2). Cells with abnormal mismatch repair function accumulate DNA replication errors, and novel microsatellite lengths are created. Impaired DNA mismatch repair facilitates insertions or deletions that can be frame-shift mutations as well as single-base mismatches that can be point mutations in coding regions. MSI allows mutations to be accumulated at many times the normal rate and facilitates MSI neoplastic progression (ref. 1; Fig. 1A).

The high mutational load in MSI tumors also creates many tumor-specific neoantigens, typically 10 to 50 times those of MSS tumors (3). Some of these neoantigens will be processed, presented on MHC, and recognized as foreign by T cells (Fig. 1A). In particular, frame-shifted proteins should be a rich source of neoantigens. This high neoantigen burden might be one explanation for the high level of tumor-infiltrating lymphocytes (TIL) and lymphocytic reaction in MSI tumors observed in many previous studies (1). MSI tumors have a better prognosis than MSS (2), and the higher level of neoantigens in MSI may contribute to better survival via more robust immunoediting of MSI (4).

In this issue of *Cancer Discovery*, Llosa and colleagues (3) analyzed primary sporadic colorectal cancer from patients free of prior chemotherapy and found that a subset of primary sporadic colorectal cancer displayed high infiltration of activated CD8^{+} cytotoxic T lymphocytes (CTL) as well as activated Th1 cells with IFNγ production and the Th1 transcription factor T-bet. Th17 or Th2 populations were not expanded. They determined that nearly all of the tumors of this subset were MSI colorectal cancer.

Despite a robust Th1/CTL microenvironment, MSI tumors are not naturally eradicated, though some incipient MSI tumors may be eradicated and never seen as colorectal cancer. Llosa and colleagues determined the expression of immune checkpoint molecules by immunohistochemistry; laser capture microdissection of TILs, stroma, and the invasive front combined with quantitative RT-PCR; and multiparameter flow cytometry of fresh tumors (3). They show that compared with MSS tumors, MSI tumors highly upregulate expression of multiple immune checkpoints, including programmed death-1 (PD-1) and cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) in TILs, stroma, and invasive front compartments; PD-1 ligand 1 (PD-L1) in TILs and stroma compartments; lymphocyte activation gene 3 (LAG-3) in TILs and invasive front compartments; and indolamine 2,3-dioxygenase (IDO) in the TIL compartment (ref. 3; Fig. 1B).

PD-1 is upregulated after T-cell activation, but declines when antigen is cleared. If antigen is not cleared, as in chronic infection or cancer, PD-1 expression remains elevated and T cells can enter a state of decreased effector function and proliferative capacity, termed exhaustion. PD-1 has two ligands, PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273). Engagement of PD-1 by PD-L1 or PD-L2 results in inhibition of T-cell immune functions, as shown by decreased production of cytokines such as IFNγ and IL2, decreased expression of cell survival proteins, altered motility and duration of interactions with dendritic cells (DC) and target cells, and altered metabolic activity. CTLA-4 is a potent inhibitory molecule that exerts its function via binding to CD80 and CD86, keeping them from delivering positive signals through CD28. LAG-3 is a CD4 homolog that binds MHC class II

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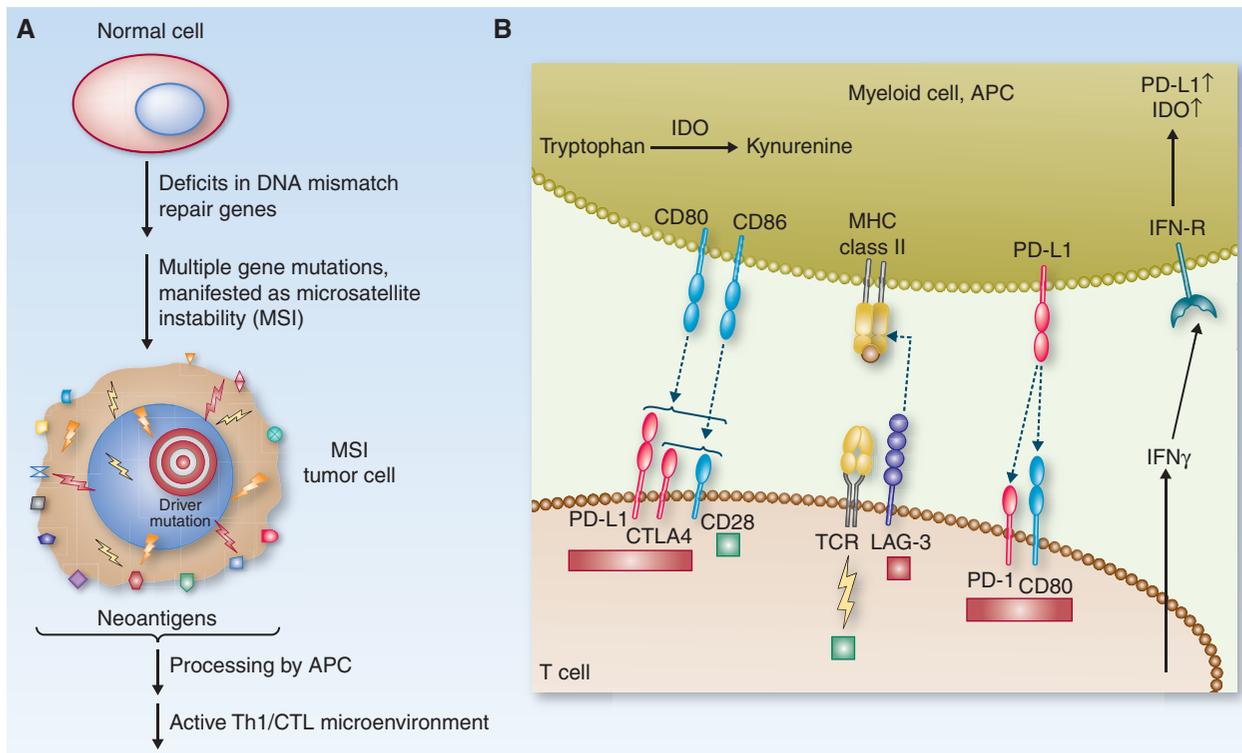©2015 American Association for Cancer Research.

Figure 1. Immune microenvironment of MSI colorectal cancer. **A,** deficits in DNA mismatch repair genes cause defective DNA mismatch repair, resulting in high levels of gene mutations manifested as MSI, which facilitate progression into an MSI colorectal cancer. Mutation-generated neoantigens are processed and presented on antigen-presenting cells (APC) and stimulate T-cell activation, leading to an active Th1/CTL microenvironment; however, (**B**) upregulation of expression of checkpoint molecules such as PD-1, CTLA-4, and LAG-3 on T cells, PD-L1 on myeloid cells, and IDO expression in myeloid cells, APCs, and tumor cells delivers inhibitory signals to suppress T-cell activation, resulting in an immunosuppressive microenvironment. Green indicates activating and red inhibitory signals.

with a higher affinity than CD4. LAG-3 is important in controlling CD8$^+$ T-cell activity within target organs. LAG-3 is also required for maximal regulatory T-cell function and can inhibit DC function by engaging MHC II on DC. IDO is an enzyme that catalyzes the degradation of the essential amino acid tryptophan to kynurenine. Production and secretion of IDO are induced by IFNγ and IL10. In the tumor microenvironment, IDO is produced by tumor cells, antigen-presenting cells (APC), tumor-associated myeloid-derived suppressor cells (MDSC), and tumor-associated macrophages (TAM) in response to these inflammatory signals. High levels of IDO reduce tryptophan levels and create kynurenine, both of which contribute to suppression of T-cell activity and are a potential mechanism for immune tolerance to tumor antigens (5).

In melanoma and many tumor types, the interface between the wave of infiltrating lymphocytes and the tumor is characterized by PD-L1 expression on the tumor cells (6). This is believed to be a consequence of T-cell recognition of the tumor leading to IFNγ expression by the T cells that in turn stimulates PD-L1 expression on the tumor cells. This IFNγ and PD-L1 expression constitutes a feedback loop, inhibiting the T-cell antitumor response via engagement of PD-1 by PD-L1. In contrast, in MSI colorectal cancer the interface between tumor and T cells was characterized by little expression of PD-L1 on the tumor cells despite INFγ expression

by the T cells. Instead, the T-cell infiltrate was interlaced with an abundant PD-L1–positive myeloid cell population (3). Presumably, these PD-L1–positive myeloid cells serve to inhibit the T-cell response. These PD-L1–positive myeloid cells were characterized as CD11b$^+$HLA–DR^{lo}CD15$^-$CD14$^+$CD33$^+$, which some investigators would call monocytic MDSC (7); however, there is not general agreement on human MDSC markers, and Llosa and colleagues did not pursue this point. They also explored the lack of PD-L1 expression on the MSI tumor cells and found that in response to IFNγ, both MSI and MSS colorectal cancer cell lines modestly upregulated PD-L1 and HLA-DR in comparison with the robust upregulation on melanoma and other cancer cell lines (3). They speculated that this might be a consequence of impaired STAT1 signaling, but changes in IFN receptor might also be involved (3).

One mystery is why the high load of neoantigens does not lead to immune eradication of MSI colorectal cancer. MSI tumors most often arise on the right side of the body in the proximal colon, and recent work has shown that the gut microbiota contributes to the development of colorectal cancer. The abundance of *Fusobacterium* species, particularly *F. nucleatum*, in tumor tissue has been shown to be associated with MSI colorectal cancer (8). *Fusobacterium* proteins can selectively recruit tumor-infiltrating myeloid cells and inhibit T-cell proliferation (9). *Fusobacterium* may facilitate upregulation of checkpoint ligands such as PD-L1 in these recruited

myeloid cells. We would speculate that *Fusobacterium* might be critically involved in suppressing the immune response at the time when MSI begins to generate a high burden of neoantigens that should facilitate immune eradication. Once the T-cell population fails to eliminate the tumor, it lapses into a chronic response with high expression of exhaustion markers and susceptibility to immunoinhibitory signals.

Anti–PD-1 and anti–PD-L1 antibodies have demonstrated significantly durable efficacy in patients with melanoma, renal cancer, and non–small cell lung cancer (3). However, colorectal cancer appeared to be a poor responder to antibody blockade of PD-1 or PD-L1 in clinical trials (3). Because most MSI colorectal cancers typically present with lower-stage disease than MSS colorectal cancers, the MSI subtype represents only 5% to 6% of the stage IV colorectal cancer population (10), and likely there were few MSI patients in PD-1 and PD-L1 clinical trials, with 18 and 19 patients with advanced colorectal cancer, respectively.

All of the five checkpoint molecules upregulated in MSI tumors are currently being targeted clinically with inhibitors (3). The FDA approved a CTLA-4 monoclonal antibody (mAb; ipilumumab) in 2010 and a PD-1 mAb (pembrolizumab) in 2014 for melanoma treatment, and multiple other pathway agents are in clinical trials and approaching approval. Multiple LAG-3 mAbs and small-molecule IDO pathway inhibitors are in development or clinical trials. On the basis of Llosa and colleagues' findings of high expression of PD-1 and PD-L1 in MSI colorectal cancer, two clinical trials have been initiated to test PD-1 blockade in patients with MSI colorectal cancer (3). Combinations with IDO, LAG-3, CTLA-4, and other checkpoints will likely follow.

This still leaves a great need for effective immunotherapies in the remaining 95% of stage IV colorectal cancer. As the checkpoint blockade molecules were discovered by their effects on immune tolerance, this suggests we need to deepen our understanding of the specialized mechanisms of gut immune tolerance to identify additional targets for colorectal cancer immunotherapy.

Disclosure of Potential Conflicts of Interest

Y. Xiao is an inventor on a patent application for PD-L2 interaction with repulsive guidance molecule b which has been licensed to Novartis. G.J Freeman is a consultant/advisory board member for Novartis and has patents and receives patent royalties on the PD-1 pathway from Bristol-Myers Squibb, Merck, Roche, EMD Serono, Amplimmune/AstraZeneca, Boehringer-Mannheim, and Novartis.

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