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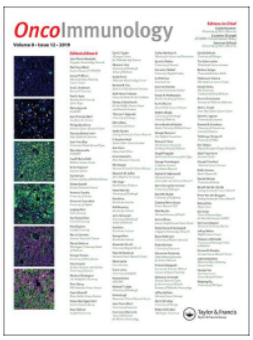

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AUTHOR'S VIEW

License to kill: microsatellite instability and immune contexture

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ABSTRACT

Colorectal cancers (CRCs) with microsatellite instability (MSI) are due to a defect in the DNA mismatch repair (MMR) system resulting in an accumulation of frame-shift mutations. They are characterized by a tumor microenvironment richer in cytotoxic CD8 T-cells (CTLs) and a better prognosis compared to microsatellite stable (MSS) CRCs. The mechanisms by which defective MMR system may influence tumor-infiltrating immune cells and their impact on patient survival were still unclear. Thus, we performed a comprehensive analysis of MSI colorectal tumors.

We found that the numbers of frame-shift mutations potentially resulting in neo-epitopes were positively correlated to the density of tumor infiltrating CD8 T-cells but were lower than expected at random. We also evidenced that MSI patients could naturally harbor CTLs targeting frame-shift mutation-derived antigens. This favors the hypothesis of an active immunosurveillance in MSI colorectal tumors leading to the genetic evidence of an immunoediting. To evaluate the link between MSI tumor immune contexture and prognosis, we took advantage of a standardized assay that we developed to quantify tumor-infiltrating T-cells, the Immunoscore. Multivariate analyses revealed an advantage of Immunoscore over MSI in predicting recurrence and survival. Our data suggests that the prognostic value of MSI could be attributed to major underlying differences of infiltrating immune cells. Immunotherapeutic treatments, that are more efficient in patients with a preexisting anti-tumor immunity, were approved in MSI patients following successful clinical trials. We suggest that the Immunoscore could be used not only for colorectal tumor prognosis but also for predicting responses to immunotherapies.

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The cancer immune contexture

The understanding of cancer and of the importance intratumor immunity has made considerable progress in the last two decades.[1] It is now clear that the immune microenvironment plays a central role regarding cancer development and patients' survival, from pre-cancer lesions to late metachronous metastases.[1,2] It was shown, for the first time in CRC that the type, density, quality and location of immune cell within the tumor site predicted patients' survival better than the classical TNM system.[1–6] This led to the powerful concept of cancer immune contexture[1,2] and to the development of an assay to measure the antitumor immune response, the "Immunoscore."[3–9] It is a standardized consensus scoring-system based on densities of two lymphocyte populations (CD3, CD8) infiltrating the tumor and invasive margin with a highly significant prognostic value in CRC.

The successes of several immunotherapies boosting this natural T-cell response against malignant cells have generated tremendous enthusiasm.[10] Notably, antibodies targeting checkpoints (CTLA-4, PD-1, PD-L1) have shown major clinical successes in multiple cancer types. However, many cancer patients are not responding to these therapies and to predict which patients will respond it is essential to implement protocols to monitor immune-related parameters. The PD-1/PD-L1 pathway represents an adaptive capacity of tumors to inhibit cytotoxic T-cells. There is a clear trend for tumors with a pre-inflamed environment rich in CD8 + T-cells and in PD-L1+ cells to respond better to anti-PD-1 treatment.

Although a majority of CRC patients do not respond to PD-1 blockade treatments, recent trials led to the approval of immunotherapies in the subset of CRC with MSI.[10] We believe that adding immune parameters, like Immunoscore, to tumor classification could improve selection of CRC patients that will benefit from these therapies.

Microsatellite instability and immune contexture

Microsatellite instability (MSI) is due to a DNA mismatch repair (MMR) system deficiency. This MMR defect results in an accumulation of insertions and deletions of nucleotides into coding repeat sequences. This can lead to frame-shift mutations that are a potential source of immunogenic neo-antigens recognized by the immune system. Strikingly, although deficient for DNA-repair genes, tumors with MSI

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are generally reported to have a more favorable outcome with reduced likelihood of metastases compared to microsatellite stable (MSS) tumors. MSI tumors are also reported to harbor more infiltrating lymphocytes. Thus, the mechanistic relationship between MSI and the anti-tumor immune response is of major interest. We performed an analysis of the genetic, genomic and immune landscape of CRC tumors,[11–13] in order to evaluate the hypothesis that the frame-shift mutations due to MMR system damage could generate immunogenic neo-peptides targeted by a T-cell response giving a survival advantage to MSI CRC patients over MSS CRC patients.

We found that MSI tumors had increased numbers of infiltrating cytotoxic T-cells, increased *in situ* proliferation of T-cells and increased numbers of frame-shift mutations (that lead to potential immunogenic neo-antigens). Also, the number of frame-shift mutations was positively correlated with the density of infiltrating CD8+ cells but not FOXP3+ cells. Using suitable algorithms, we predicted which mutations, within the whole exome of each individual, would give rise to antigenic neo-peptides presented in the HLA class I context. These *in silico* predictions revealed that the frequency of mutations resulting in neo-epitopes was lower than expected at random. Thus, we concluded from this genetic evidence that human CRCs are prone to a negative selection of antigenic tumor variants (i.e. an immunoediting), in particular, for point mutations and frameshift mutations in MSI patients.

To test the reactivity of MSI CRC patient cytotoxic T-lymphocytes (CTLs) against tumor-specific frame-shift mutation-derived neopeptides, we stimulated *in vitro* peripheral T-cells from healthy donors and CRC patients with artificial antigen presenting cells. Neopeptide-specific CTLs could only be obtained from MSI CRC patients harboring the corresponding frame-shift mutations in their tumor. Frame-shift mutations in *ASTE1, HNF1A* genes[12] and *TGFRB2* gene[13] were associated with anti-frameshift mutation CTLs.

These functional anti-frameshift mutation CTLs were able to kill *in vitro* MSI tumor cell lines. This suggested that MSI CRC patient immune cells had previously encountered these peptides *in vivo* and developed a specific reaction against them. Moreover, such frameshift mutation-specific CTLs were, for the first time, visualized *in situ*.[13]

The genetic and genomic landscape in CRC patients revealed significant differences in mutation patterns, chromosomal instability and gene expression between MSI and MSS CRC tumors.[13] Whole-genome expression changes, revealed a prominent increase in expression of immune-related genes, including chemokine, cytokine, type 1 helper and cytotoxic T-cells, in MSI tumors. However, a subgroup of MSS tumors also expressed high levels of these genes, which correlated with prolonged survival. We further investigated the dependency or independency of the MSI and Immunoscore parameters with regards to the patient survival. Importantly, among MSI patients, only the ones with high Immunoscore had a prolonged survival. We demonstrated a statistical dependence between the MSI status and the immune criteria, with a superiority of the Immunoscore, as it also predicted outcome in MSS patients.[13] Thus, assessment of the immune status using Immunoscore provides an indicator of tumor recurrence beyond MSI.

Our study demonstrated that strong and effective anti-tumor immunity may naturally be elicited against true tumor-specific antigens resulting from somatic mutations (Figure 1) and that Immunoscore should better define the prognosis of CRC patients, better identify patients at high-risk of tumor recurrence regardless of MSI status, and help to stratify patients who will likely benefit from immunotherapies.

Conclusion and implications

The good prognosis of MSI compared to MSS CRCs could be attributed to major differences of density and quality of infiltrating immune cells. As MSI patients generally present natural high cytotoxic T-cell responses, they are prone to respond efficiently to immunotherapy approaches, as recently illustrated in clinical trials boosting T-cell responses with anti-CTLA-4, anti-PD-1 and anti-PD-L1.[10] Our data would argue, 1) that MSI patients at early stage may benefit the most from checkpoint T-cell therapies, as they have strong effector T-cell response, and present more frequently with a high Immunoscore, and 2) that among metastatic MSI patients, only the subgroup with a high Immunoscore may benefit from checkpoint T-cell therapies. Importantly, the consensus Immunoscore has now been introduced into cancer classification (WHO classification of Digestive System Tumors) and into clinical guidelines (ESMO).[1,2] We strongly believe that immunoscore could be a good tool to select patients responding to checkpoint immunotherapy, including within the MSI subgroup.[10]

Furthermore, there are broad practical contributions of this study 1) for cancer vaccines in terms of new and exciting possibilities for using hot-spot neo-antigens, 2) for personalized medicine in terms of biomarker development and 3) for understanding immune escape in cancer. The possibility of using vaccines as anticancer agents and recent advances in the development of personalized neoantigen-based therapeutic cancer vaccines has been thoroughly discussed.[14–21] Our study caught immunosurveillance in the act and give important clues on how to manipulate the immune system for better therapeutic options.

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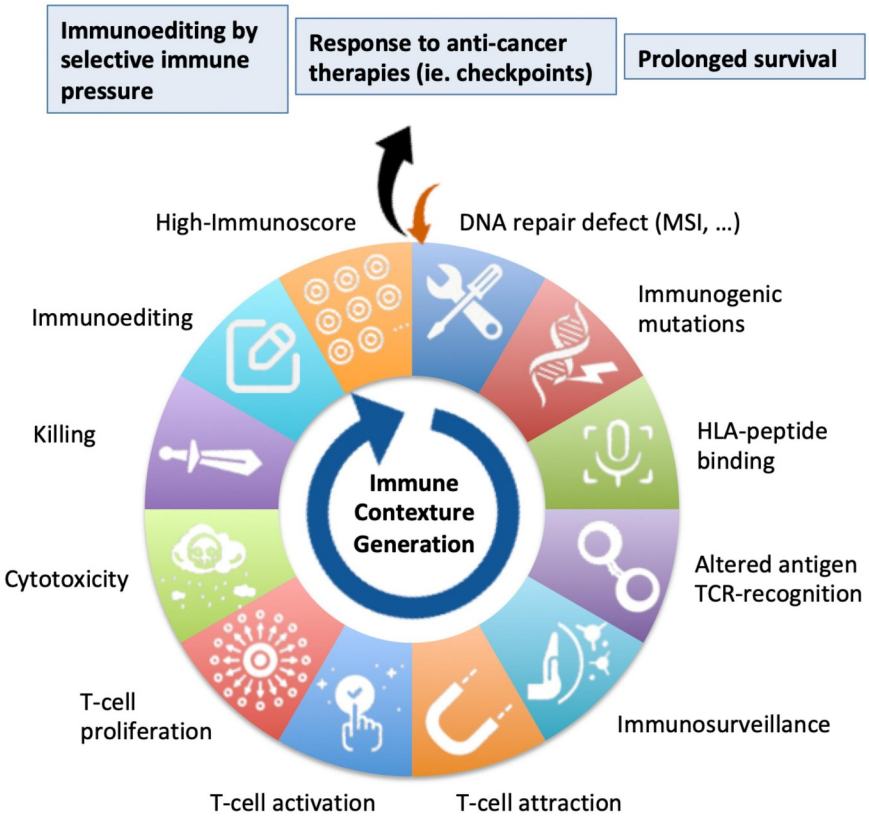

Figure 1. DNA repair defect, tumor immune contexture and prognosis. Colorectal cancers (CRCs) with microsatellite instability (MSI) are due to a defect of the DNA mismatch repair (MMR) system that leads to an accumulation of insertions and deletions of nucleotides into repeat sequences. When such mutations occur in coding sequences, reading frames of the genes can be shifted and neo-antigens synthetized and presented at the surface of the mutated tumor cells, as neo-peptides bound to HLA-I molecules. These antigens can be considered as non-self and elicite immune reactions. T-cells can then be attracted to the tumor site and stimulated for proliferation and cytotoxic activity. Tumor cells harboring such immunogenic mutation can then be exposed to a selective immune pressure, the tumor immunoediting. Such anti-immune responses, characterized by high immune infiltrations (high Immunoscore) are associated with better clinical outcomes and responses to immunotherapies.

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Declaration of interests

JG and BM have patents associated with the immune prognostic biomarkers. JG is co-founder of HalioDx biotech company. Immunoscore® a registered trademark from the National Institute of Health and Medical Research (INSERM) licensed to HalioDx.

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