Critical impact of tumor immune response on cancer patient prognosis is now widely recognized. Numerous studies have shown that a dense infiltration of memory and CD8⁺ T lymphocytes is associated with an improved survival of CRC patients. CRCs with microsatellite instability (MSI) represent 15% of all CRCs, including Lynch syndrome, the most frequent hereditary form of this disease. MSI-CRCs are now well known to be associated with a better prognosis and to have a higher density of tumor-infiltrating lymphocytes (TILs) than microsatellite stable (MSS)-CRCs. In particular, several immunohistochemistry studies have revealed an especially high infiltration of intraepithelial activated CD8⁺ cells within MSI colorectal tumors.

MSI is due to a defect in the mismatch repair (MMR) system, involved in DNA replication fidelity. During replication, DNA polymerase sometimes integrates a wrong number of nucleotides within repeat sequences but these mistakes are normally detected by the MMR machinery to be repaired. In the absence of a functional MMR system, tumor cells accumulate insertions and deletions within repeat sequences. In coding sequences, such mutations can lead to shifts in open reading frames and to synthesis of neoantigens fragmented into neoepitopes, presented in the HLA-I context by mutated tumor cells. As these frameshift mutation (FSM)-derived neopeptides are truly tumor-specific, they are less prone to self-tolerance mechanisms. Therefore, it has long been suspected that the high density of CD8⁺ cells in MSI-CRCs was due to an accumulation of FSM-derived neoantigen-specific T lymphocytes. However, the clear link between CD8⁺ cells infiltrating MSI tumors and FSMs resulting in neoantigen expression was missing. This prompted us to study the relationship between TIL densities and tumor-specific FSMs in MSI-CRC patients.

To detect FSMs, we designed multiplex PCRs amplifying 22 coding repeat sequences frequently mutated in MSI-CRCs and possibly involved in cancer development. In parallel, within the same tumors, we quantified TIL subpopulation densities by immunohistochemistry. In a previous study, our team showed that, in MSI-CRCs, total (CD3⁺) TIL density was significantly higher when the total number of FSMs detected in the tumors was above the median. In a more recent studies, we showed that FSM total number was positively correlated with the density of CD8⁺ (but not FOXP3⁺) cells infiltrating these tumors, and we even more precisely defined the immune signature of MSI-CRCs. Furthermore, we showed that ASTE1, HNF1A or TCF7L2 gene FSMs were associated with a significantly higher CD8⁺ TIL density, which further increased when at least one of these genes was mutated in all tumor cells. These results establish, for the first time to our knowledge, a direct link between FSMs and CD8⁺ T cell infiltration in MSI-CRCs.

To test the reactivity of MSI-CRC patient T lymphocytes against tumor-specific FSM-derived neopeptides, we in vitro stimulated peripheral T lymphocytes from healthy donors and CRC patients with artificial antigen presenting cells (AAPCs) generated in the laboratory. These AAPCs are NIH/3T3 fibroblasts genetically engineered to efficiently present a peptide of interest in the HLA-I context. Neopeptide-specific cytotoxic T lymphocytes could only be obtained from MSI-CRC patients harboring the corresponding FSMs in their tumor. This suggests that MSI-CRC patient immune cells had previously encountered these peptides in vivo and developed a specific reaction against them. Overall, our results reinforce the hypothesis according to which FSMs can result in the production of...
immunogenic neopeptides leading to the accumulation of tumor-specific CD8+ T lymphocytes in MSI tumors. Nevertheless, despite the large quantity of intratumoral CD8+ T cells, MSI-CRCs that arise have not been eliminated by an effective antitumor immune response. It appears that an equilibrium is reached between these tumors and the immune system. Without any treatment, the balance tends to progressively shift in favor of the malignant cells to the detriment of host immunity. Recently, a study by Llosa et al. showed that, compared to MSS-CRCs, MSI-CRCs expressed a higher level of several immune checkpoint related proteins, including PD-1, PD-L1, CTLA-4, LAG-3 and IDO.8 In agreement with these results and our findings, it can be hypothesized that MSI-tumors contain abundant FSM-derived neoantigen-specific CD8+ T cells, but that the cytotoxic activity of these TILs is strongly counterbalanced by multiple inhibitory signals. As a consequence, MSI-CRC patients could be particularly good responders to immunotherapeutic procedures. Indeed, PD-1 signaling blockade, which had provided very disappointing results for CRC patients to date, has recently been successfully tested in MSI-CRCs with impressive results.9 We can expect that other immune checkpoint blocking antibodies will also soon be successfully tested in MSI patients. Furthermore, specific immunotherapy strategies (as vaccination or adoptive cell transfer) targeting FSM-derived neoantigens could also be promising for MSI-CRC patients. Our results underline the feasibility of developing personalized cellular adoptive immunotherapy strategies based on tumor mutation profiling and in vitro stimulation of autologous tumor-specific cytotoxic T lymphocytes.

In conclusion, the positive correlations we found between FSMs and CD8+ cell density could represent a missing piece of the puzzle to ascertain that the high density of CD8+ TILs which characterize MSI-CRCs is due to the immunogenicity of the FSM-derived neoantigens. Overall, our results, in agreement with other recent data discussed here, suggest that MSI-CRCs might have an immune microenvironment rich in tumor neoantigen-specific CD8+ T cells with an insufficient effective activity, notably due to strong inhibitory signals. Immunotherapy approaches could successfully brake tolerance and consequently improve MSI cancer treatment, especially in young Lynch syndrome patients (Fig. 1).

Disclosure of potential conflicts of interest

Jérôme Galon is co-founder of HalioDx company.

References

1. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12:298-306; PMID:22419253; http://dx.doi.org/10.1038/nrc3245
2. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol Off J Am Soc Clin Oncol 2005; 23:609-18; PMID:15659508; http://dx.doi.org/10.1200/JCO.2005.01.086

Figure 1. How CD8+ T lymphocytes could target tumor-specific frameshift mutation-derived neoantigens. (A) MSI tumors are prone to insertions and deletions in repeat sequences. In coding repeat sequences (as illustrated here with the 10 adenine tract within TGFBR2 gene, first coding repeat sequence shown by Linnebacher M. et al. to be involved in the production of an immunogenic frameshift peptide10), this can lead to a shift in the open reading frame and to the synthesis of a neoprotein expressed only in tumor cells harboring this mutation. Intracellular degradation of this neoantigen can release immunogenic neopeptides presented on HLA-I molecules by mutated tumor cells. Contrary to self peptides subjected to self-tolerance, neopeptides are more likely to be recognized by specific CD8+ T lymphocytes which could accumulate within MSI-tumor nests as frameshift mutations accumulate within tumor cells. Nevertheless when intratumoral T lymphocyte activity is dampened, by immune checkpoint inhibitory signals for instance, tumor cells fail to be eliminated. (B) Immunotherapy strategies, favoring active tumor-specific immune responses, could be highly beneficial in the context of MSI-CRCs. PD-1 pathway-blockade (1) has already shown very promising results. Other strategies could be of interest, like adoptive transfer of autologous tumor-specific cytotoxic T lymphocytes previously stimulated in vitro, e.g., with artificial antigen presenting cells (2) or cancer vaccination.
3. Michael-Robinson JM, Biemer-Hüttmann A, Purdie DM, Walsh MD, Simms LA, Biden KG, Young JP, Leggett BA, Jass JR, Radford-Smith GL. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. Gut 2001; 48:360-6; PMID:11171826; http://dx.doi.org/10.1136/gut.48.3.360

4. Phillips SM, Banerjea A, Feakins R, Li SR, Bustin SA, Dorudi S. Tumour-infiltrating lymphocytes in colorectal cancer with microsatellite instability are activated and cytotoxic. Br J Surg 2004; 91:469-75; PMID:15048750; http://dx.doi.org/10.1002/bjs.4472

5. Tougeron D, Fauquembergue E, Rouquette A, Le Pessot F, Sesboüe R, Laurent M, Berthet P, Mauillon J, Di Fiore F, Sabourin JC et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. Mod Pathol Off J U S Can Acad Pathol Inc 2009; 22:1186-95; PMID:19503063; http://dx.doi.org/10.1038/modpathol.2009.80.

6. Maby P, Tougeron D, Hamieh M, Mlecnik B, Kora H, Bindea G, Angell HK, Fredriksen T, Elle N, Fauquembergue E et al. Correlation between Density of CD8+ T-cell Infiltrate in Microsatellite Unstable Colorectal Cancers and Frameshift Mutations: A Rationale for Personalized Immunotherapy. Cancer Res 2015; 75:3446-55; PMID:26060019; http://dx.doi.org/10.1158/0008-5472.CAN-14-3051

7. Latouche JB, Sadelain M. Induction of human cytotoxic T lymphocytes by artificial antigen-presenting cells. Nat Biotechnol 2000; 18:405-9; PMID:10748520; http://dx.doi.org/10.1038/74455

8. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Luber BS et al. The Vigorous Immune Microenvironment of Microsatellite Instable Colon Cancer Is Balanced by Multiple Counter-Inhibitory Checkpoints. Cancer Discov 2015; 5:43-51; PMID:25358689; http://dx.doi.org/10.1158/2159-8290.CD-14-0863

9. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 2015; 372:2509-20; PMID:26028255; http://dx.doi.org/10.1056/NEJMoa1500596

10. Linnebacher M, Gebert J, Rudy W, Woerner S, Yuan YP, Bork P, von Knobel Doeberitz M. Frameshift peptide-derived T-cell epitopes: a source of novel tumor-specific antigens. Int J Cancer J Int Cancer 2001; 93:6-11; PMID:11391614; http://dx.doi.org/10.1002/ijc.1298

11. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, Church SE, Lafontaine L, Fischer M, Fredriksen T et al. Integrative analysis of colorectal cancer shows Immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 2016; in press