Exhausted Markers in Cutaneous T-Cell Lymphoma: The Face that Launched a Thousand Ships

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Immune-modulatory therapies are widely appreciated to rejuvenate host antitumor immunity and improve mortality in solid-organ cancers. Targeting the exhausted markers such as PD-1, CTLA4, TIM3, LAG3 are particularly attractive owing to the activation of the immune response. However, their role in cutaneous T-cell lymphomas is less defined owing to the expression of those exhausted markers on both nonmalignant and malignant lymphocytes. In a new article of the Journal of Investigative Dermatology, Han et al. (2021) showed that microRNAs in malignant T cells could regulate the expression of PD-1, CTLA4, TIM3, and LAG3 and simultaneously mediate evasion from immune surveillance. These findings get us one step closer in our further investigation of whether those molecules could be targeted therapeutically.


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The evasion of cellular destruction by the host immune response is essential for tumor growth and a hallmark of cancer (Hanahan and Weinberg, 2011). Central to escaping immunologic surveillance is the hijacking of immune checkpoint pathways that were intended to enforce self-tolerance and quiescence among activated T cells to avert autoimmunity. In turn, the unaltered success of immune checkpoint blockade in treating inoperable end-stage solid-organ tumors has fomented an explosive interest in this therapeutic modality. Antibody-mediated blockade of the inhibitory receptors PD-1 and CTLA4 that reinvigorates exhausted tumor-infiltrating lymphocytes have shown efficacy and awarded Food and Drug Administration approval for melanoma, head and neck squamous cell carcinomas, small and nonsmall cell lung cancer, renal cell carcinoma, and colorectal cancers (Vaddepally et al., 2020). However, the broader therapeutic applicability of these immune stimulatory therapies to cutaneous T-cell lymphomas (CTCLs) has remained less clearly defined because malignant T-cell clones also express inhibitory checkpoint molecules. Thus, elucidating how coinhibitory receptors are regulated among malignant T-cell clones may help to guide the current and potentially novel immunotherapies. In a new article of the Journal of Investigative Dermatology, Han et al. (2021) identify an essential role for microRNAs (miRNAs) in regulating cellular exhaustion and averting tumor surveillance among neoplastic clones in patients with mycosis fungoides (MF) and Sézary syndrome.

Immunoregulatory receptors in MF and Sézary syndrome

Investigations into the role of immunoregulatory receptors in MF and Sézary syndrome are sparse. This has generated a clear knowledge gap in understanding how antitumor immunity can be bolstered in these malignancies. Within this context, Han et al. (2021) present timely mechanistic insight into how miRNAs regulate immune-modulatory receptors in MF and Sézary syndrome.

miRNAs are noncoding single-stranded RNA molecules of 18–22 nucleotides that safeguard key
biological processes involving cell proliferation, differentiation, and apoptosis under homeostatic physiological conditions. As a result of their crucial role in regulating critical cellular functions, dysregulation of miRNAs is often observed during cellular transformation, and signature patterns of miRNA expression have been identified in numerous solid organ and hematologic malignancies (Calin and Croce, 2006). In this study, three miRNAs (miR-155-5p, miR-130b-3p, miR-21-3p) from lesional skin were found to be increasingly expressed with MF progression (i.e., from patches to plaques to tumors) and associated with upregulation of the coinhibitory molecules PD-1, PD-L1, and TIM3 (Figure 1a). In turn, protein expression of these surface receptors was significantly reduced after silencing of miR-155-5p, miR-130b-3p, and miR-21-3p among MyLa and HuT78 cell lines. Furthermore, CD8+ T-cell–mediated cellular destruction of MyLa and HuT78 cells in vitro was unleashed after miRNA silencing that paralleled the upregulation of CD107a expression and IFN-γ secretion by cytotoxic cells. The ability of miRNAs to prevent effector T-cell–mediated cytolysis suggests that this may be a pathway exploited by malignant cells to avoid immune surveillance.

In regard to erythrodermic MF and Sézary syndrome, miR-155-5p, miR-130b-3p, and miR-21-3p were...

**Clinical Implications**

- Three microRNAs (miRNAs) were identified to be progressively upregulated with advancing the stage of mycosis fungoides.
- miRNA expression is associated with an upregulation of coinhibitory receptors among malignant CD4+ T cells.
- miRNA silencing unmasks malignant CD4+ T cells to allow elimination by cytotoxic effectors.

Figure 1. Tumor microenvironment in MF and the role of miRNAs in the regulation of exhaustion receptors. (a) Regulation of coinhibitory surface receptors and evasion of immune surveillance by miRNAs in MF and Sézary syndrome. Three miRNAs (miR-155-5p, miR-130b-3p, and miR-21-3p) are increasingly expressed with MF progression (i.e., from patches to plaques to tumors) and are associated with the upregulation of the coinhibitory molecules PD-1, PD-L1, and TIM3. In Sézary syndrome, malignant cells are still enriched for exhaustion markers; yet, miRNA expression is on par with that of healthy skin, implicating an unknown secondary role for miRNAs in Sézary syndrome. (b) Multispectral imaging shows a complex interplay of various cellular components of MF tumor microenvironment. Patch MF ×20. (c) Components of the cutaneous tumor microenvironment in the patch/plaque stage of MF. MF, mycosis fungoides; miRNA, microRNA; Th, T helper type.
downregulated relative to those in tumor-stage MF, with most miRNAs expression on par with that of healthy skin. If these miRNAs are responsible for inducing the expression of costimulatory molecules, malignant cells in leukemic CD4+ CTCL would be expected to lose their exhausted phenotype. However, because PD-1 is near ubiquitously expressed in Sézary syndrome, the loss of miRNAs in advanced-stage CTCL likely reflects their involvement in other key regulatory roles. The advent of high-throughput TCR sequencing has provided strong evidence that clonal MF and Sézary cells stem from neoplastic cells that seed the skin (Iyer et al., 2019). Therefore, a tantalizing hypothesis to reconcile these findings would be that the identified miRNAs not only regulate exhaustion and survival but also mediate cellular trafficking of neoplastic clones, with subsequent loss of miRNAs ablating the skin homing that subsequently causes malignant cells to accumulate in the peripheral blood as seen in erythrodermic MF or Sézary syndrome. A direct therapeutic corollary of this hypothesis would be that whereas silencing miRNAs would be useful in treating patch, plaque, and tumor-stage MF, adding back miRNAs may mediate the reversal and downstaging of erythrodermic MF or Sézary syndrome.

**Immune-modulatory agents in MF and Sézary syndrome**

The findings by Han et al. (2021) illustrating that miRNAs are upregulated in parallel with tumor stage, along with their ability to abet in averting detection by the host immune system, highlight exciting new therapeutic avenues for CTCL, particularly because the potency of immune checkpoint blockade has been less consistent for this disease. A phase I trial using the anti–PD-1 mAb nivolumab elicited a partial response among only 15% of patients (2 of 13) with MF (Lesokhin et al., 2016). More impressive results were reported in a phase II trial using the PD-1 inhibitor pembrolizumab, which showed ~8% complete response and 30% partial response in patients with MF (Khodadoust et al., 2020). These discrepancies in PD-1 blockade efficacy may be attributed to differences in epitope-paratope binding. Nivolumab binds the PD-1 N-terminal loop, whereas pembrolizumab targets the PD-L1–binding IgV domain of PD-1 receptor. Nevertheless, when considered in the context of reports showing a paradoxical rapid lymphoma progression after anti–PD-1 therapy (Rauch et al., 2019), these observations suggest that this immune checkpoint pathway may not be the most optimal target, particularly for early-stage MF.

In parallel with checkpoint inhibition, emerging interest has developed around targeting CD47, a “don’t eat me signal” that is upregulated in human cancers and inhibits phagocytosis through the ligation of SIRPα expressed on phagocytes. A phase I clinical trial (ClinicalTrials.gov identifier NCT02890368) that is near complete is evaluating the safety and efficacy of intralesional injections of anti-CD47 antibody (TTI-621) in patients with relapsed and refractory tumor-stage CTCL (Querfeld et al., 2017). By employing intralesional injections, systemic toxicities may be avoided because CD47 is ubiquitously expressed at low levels among normal cells. Initial reports are promising, with all patients experiencing reductions in tumor size after a single intratumoral dose, with no patient experiencing an adverse event greater than grade 2. Depending on the results of this study, it may be possible that intralesional CD47 blockade becomes a favored modality for CTCL, whereas PD-1 inhibition is preferentially administered for solid-organ malignancies.

**Emerging therapeutic landscape in CTCL**

The cutaneous tumor microenvironment in MF is highly complex, involving perpetual crosstalk among malignant cells, stromal cells, and immune cells (Figure 1b and c). Pre-existing and/or acquired mutations in the tumor microenvironment mediate resistance to immune-modulatory therapies. Uncovering factors that predispose to drug failure were previously only possible by identifying genetic mutations or shifts in protein expression among malignant tissue en masse. For example, defects in IFN-receptor signaling and antigen presentation were identified through whole-exome sequencing among melanomas refractory to PD-1 blockade (Zaretsky et al., 2016). More recently, the development and application of multiplex immunofluorescent technology have allowed for visualization of an additional spatial dimension, which would be otherwise undetected and ignored, in understanding how the tumor microarchitecture dictates outcomes to therapy. The broader applicability of this approach to predicting therapeutic susceptibility for cutaneous T-cell malignancies is lacking and represents fertile grounds for future work that identifies patient characteristics that optimize treatment with miRNAs, intralesional CD47 blockade, and/or systemic PD-1 inhibition among patients with CTCL.

Valuable lessons from immunotherapy in metastatic melanoma have illustrated the importance of combination therapy to prevent drug resistance and overcome clonal heterogeneity. The increasing appreciation that neoplastic clones in CTCL exhibit heterogeneity to the same degree observed for solid malignancies strongly urges an investigation into combination therapies. The benefits of dual therapy have been applied before the appreciation of checkpoint inhibition, where the efficacy of psoralens and UVA light was unleashed in the presence of intramuscular recombinant IFN-α-2a (Roenigk et al., 1990). These synergistic observations likely stemmed from the ability of IFN-1s to stimulate host antitumor immunity. Future investigations into how IFN-1s may potentiate miRNAs, intralesional CD47 blockade, and/or systemic PD-1 inhibition in CTCL represent important future directions.

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**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**REFERENCES**


