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Journal

Trends in Immunology, 43(7)

ISSN

1471-4906

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Publication Date

2022-07-01

DOI

10.1016/j.it.2022.05.003

Peer reviewed

HHS Public Access

Author manuscript

Trends Immunol. Author manuscript; available in PMC 2023 July 01.

Published in final edited form as:

Trends Immunol. 2022 July ; 43(7): 500–502. doi:10.1016/j.it.2022.05.003.

Finding your niche: immune evasion in quiescent tumor reservoirs

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Abstract

Emerging immunotherapies offer a new hope for cancer patients but are not always effective even when a tumor is recognized by the immune system. Baldominos and colleagues address this challenge by characterizing a resilient niche of metabolically unique quiescent cancer cells that resist T cell mediated control.

Checkpoint blockade-based immunotherapies (CBI), such as those involving blockade of the T cell inhibitory receptor program-death 1 (PD-1) or its ligand (PDL-1), can not only extend cancer patient lives, but can also offer long-term remission [1]. The core principle of CBI is restoring hypofunctional or "exhausted" $CD8^+$ T cells (Tex), which are characterized by elevated expression of inhibitory receptors such as PD-1, contributing to decreased $CD8^+$ T cell cytokine production and cytotoxic function [2]. Tex are a heterogenous population, including terminally exhausted (Tex^{TERM}) and progenitor (Tex^{PROG}) subsets [2]. Among these, Tex^{PROG} generate all other Tex populations and are essential for the success of CBI [2], which is not always observed [1]; therefore, it is essential to understand how tumor cells may resist CBI in some patients.

One mechanism by which tumors subvert CBI is by evading $CD8^+$ T cell recognition via downregulation of tumor-targeted antigens or components of the antigen presenting machinery [1]. To explore other mechanisms of CBI evasion independently from tumor antigen downregulation, Baldominos *et al.* [3] engineered a mouse model of triple negative breast cancer (TNBC) expressing GFP and transferred TCR-transgenic GFP-specific $CD8^+$ T cells (Jedi) into these mice [4]. Thus, GFP served as both a tumor marker and a T cell antigen [3]. To model T cell responses during CBI in this mouse tumor model, the authors used $PD1^{-/-}$ Jedi T cells, which killed many, but not all, GFP^+ cells upon transfer. Importantly, the authors confirmed that the surviving GFP^+ cells did not downregulate their antigen presenting machinery, indicating that the tumor resistance strategy in this model was different from previously characterized CBI evasive mechanisms involving antigen loss or downregulated antigen presentation.

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To determine how resistant GFP^+ cells evaded GFP-specific Jedi T cell killing, the authors compared the transcriptome of surviving GFP^+ tumor cells with GFP-deficient tumor cells (the latter not targeted by Jedi T cells). This comparison revealed a signature of reduced GFP^+ cell proliferation. This was confirmed with a known reporter of quiescence (p27K-venus) and identifying specific tumor niches containing quiescent cancer cells (QCC) with low proliferation and decreased T cell infiltration [3]. Further investigation of the transcriptional state of these QCC identified signatures of hypoxia and glycolysis, described as a “Hif1a signature”. This was consistent with enhanced glucose uptake in QCC and high levels of hypoxia within their reservoirs. Notably, while QCC were non-proliferating *in situ*, they exhibited a signature of increased stemness and were more efficient than their non-quiescent counterparts in establishing tumors upon transfer into recipient mice. Remarkably, when the authors looked at tumor biopsies from TNBC patients, they detected reduced T cell infiltration in regions that expressed the quiescent marker p27 compared to controls. Consistent with previous work [5], human TNBC tumors that were enriched in a proliferation-associated pathway were also more likely to benefit from CBI, supporting the putative contribution of QCC to CBI resistance in humans [3].

Baldominos and colleagues reported low, but not absent, immune infiltration into QCC niches. Therefore, they investigated why immune cells infiltrating these niches were unable to clear QCC. To this end, the authors used a spatially resolved single cell (sc)RNA-seq method named **P**hoto-activation of **A**reas to **D**issect **M**icro**E**nviroments (PADME-seq). This approach used mice expressing the Kaede protein, known to trace photo-activated cells via fluorescence changes from green to red when stimulated with a 421nm laser. Activating this Kaede fluorescent switch in cells within or outside p27K-high QCC niches, the authors compared cellular transcriptomes via scRNA-seq. This elegant strategy revealed that QCC-niches contained: i) conventional dendritic cells (cDCs) with reduced antigen presenting machinery and a low interferon (IFN) signature, ii) suppressive fibroblasts similar to a subset previously associated with poor outcomes after CBI [6], and iii) proportionately more Tex^{TERM} than Tex^{PROG}. Overall, this showed that QCC niches were not only less infiltrated with immune cells, but that immune cells not infiltrating these reservoirs were significantly compromised [3].

Finally, the authors investigated whether the “Hif1a signature” of QCC niches was functionally related to their capacity for immune evasion. They engineered GFP^+ tumor lines expressing a constitutively-active Hif1a, or deficient for Hif1a. After transfer of Jedi T cells into these mice, Hif1a-deficient tumors showed improved growth control, while those with enforced Hif1a activity exhibited a larger proportion of T-cell-resistant tumor cells. Importantly, enforced Hif1a activity in tumor cells was sufficient to reduce the expression of antigen-presenting MHC-II in cDCs and to decrease both $CD8^+$ T cell infiltration and the proportion of Tex^{PROG} present. The converse was seen when tumor cells were Hif1a-deficient. Together, these results support the idea that Hif1a in tumor cells, at least in this model, can promote an immunosuppressive microenvironment with compromised cDCs and limited numbers of Tex^{PROG}. As Tex^{PROG} are essential for driving post-CBI-driven expansion of T cells [2] their relative depletion in QCC niches might contribute to QCC resistance to CBI [3] (Figure 1).

Baldominos and coworkers made a key observation in that QCC exhibited increased avidity for glucose and enhanced hypoxic and glycolytic gene signatures, suggesting that these cells might be engaging anaerobic glycolysis. It is therefore reasonable to hypothesize that QCC might efficiently deplete glucose and simultaneously secrete high concentrations of lactate into their environment. This might help explain the observed immune dysregulation in QCC niches, given that cDCs and T cells rely on glycolysis for antigen presentation and maintenance of effector function, respectively, and their activities can be inhibited by lactate [7]. Furthermore, immunosuppressive regulatory T cells can maintain function in low glucose and high lactate environments [8], and this might also contribute to the observed immunosuppression in QCC niches [3] (Figure 1).

Beyond the role of QCC in contributing to drive T cell and DC inhibition, the hypoxic environment itself might also favor immunosuppression. While hypoxia alone is known to enhance $CD8^+$ T cell effector function, Baldominos *et al.* could not recapitulate the MHC-II downregulation through hypoxic DC cultures, hypoxia within the complex tumor microenvironment might still be a factor to directly disable some aspects of anti-tumor immunity. It is possible, for example, that the hypoxic environment might be more favorable for Tex^{TERM} than Tex^{PROG}, known to consume high concentrations of oxygen [9]. Additionally, the reduced IFN signature in cDCs suggests lower IFN production within QCC-niches, which might be associated with reduced plasmacytoid dendritic cell (pDC) function – cells known for their outstanding type-I IFN production capacity but expected to be disabled in hypoxic environments. Indeed, pDCs must engage glucose consumption and oxidative metabolism to support IFN-I synthesis and are highly susceptible to lactate inhibition [10]. Finally, beyond hypoxia, putatively altered chemokine composition might also contribute to reduced immune infiltration in QCC niches.

In conclusion, using a combination of classic methods and cutting-edge spatial transcriptomics, Baldominos and colleagues identified a QCC niche that evaded CBI despite seemingly unaltered antigen presentation. The discovery of QCC-niches as resisting T cell killing is unfortunate because QCC have previously been established to resist other anti-tumor treatments (e.g. chemotherapy and radiation) [11] – an endurance that might also be connected to their capacity to suppress immune responses. In the future, it will be important to disentangle the contribution of the aforementioned mechanisms to immunosuppression within QCC-niches and the extent to which correcting one or more of these axes might aid the eradication of QCC. Thus, the study by Agudo's group [3] has paved the way to address this question and potentially uncover new strategies for improving CBI efficacy.

ACKNOWLEDGMENTS

We thank members of the Zuniga lab for fruitful discussions. As space was limited, we were unable to cite all available studies, and so apologize to any authors who feel their studies were not adequately represented in our accounting. Lymphoidstem-cell, dendritic-cell-2, emptycell-3d-3, and emptycell-5 icons by Servier https://smart.servier.com/ are modified and licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/. Research in the Zuniga lab is supported by NIH grants AI145314, AI081923, AI113923 and AI132122.

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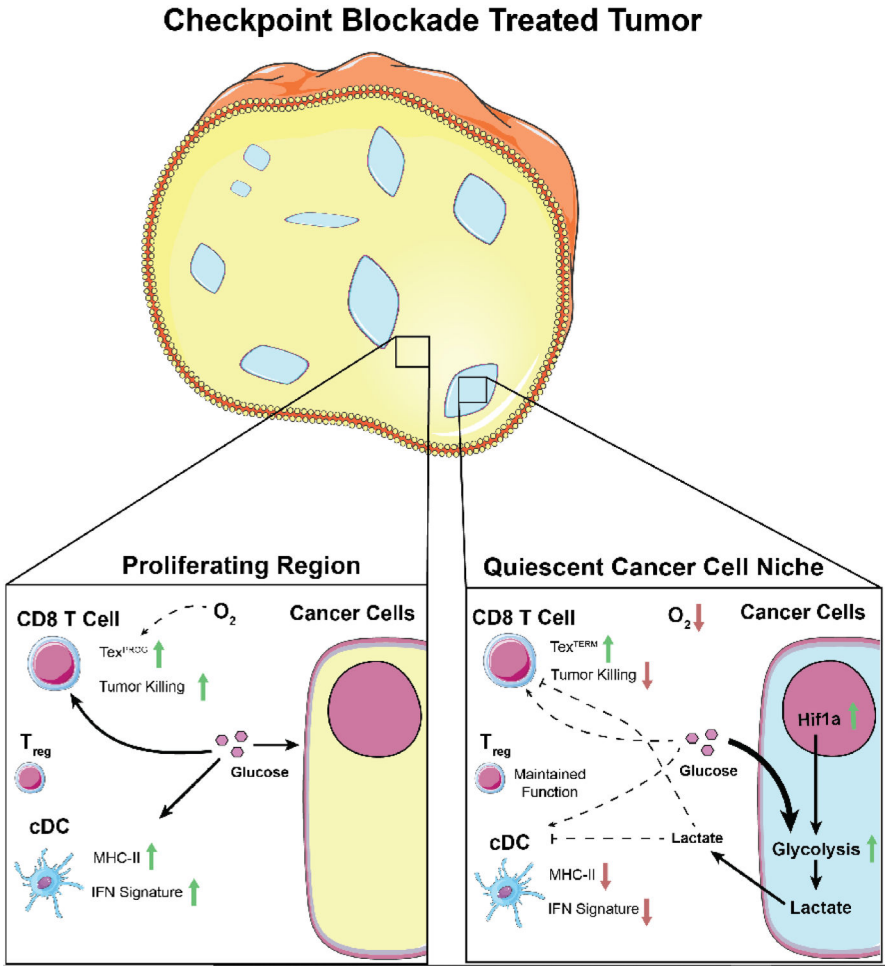

Figure 1. Quiescent cancer cell niches can suppress immune function.

Breast cancer tumors can develop internal niches of proliferating (yellow, left) and quiescent (blue, right) cancer cells (QCC). QCCs show signs of higher Hif1a activity signatures, increased propensity for glucose uptake, and up-regulated expression of glycolytic enzymes with respect to their proliferating counterparts [3]. QCC regions show low $CD8^+$ T cell infiltration with skewing towards the generation ofTex^{TERM} and away from Tex^{PROG}, as well as the presence of conventional dendritic cells (cDCs) with a decreased MHC-II and IFN signature. This immunosuppressive environment is associated with QCC resistance to T cell killing [3]. We speculate that glucose depletion and potential lactate release by the glycolytic QCC, as well as a direct effect of the hypoxic environment on immune cells might contribute to immunosuppression within QCC-niches. Tex^{TERM}, terminally exhausted T cell; Tex^{PROG}, progenitor exhausted T cell; cDC, conventional dendritic cell; Treg, regulatory T cell.