Regulatory T Cells Keep Pancreatic Cancer at Bay

Berk Akyut¹, Ruonan Chen¹, and George Miller¹²

Summary: Although CD4⁺ FOXP3⁺ T regulatory (Treg) cells are well-known mediators of immunologic tolerance, their influences in the tumor microenvironment are incompletely understood. Writing in this issue of Cancer Discovery, Zhang and colleagues demonstrate that in pancreatic cancer, Treg cells promote the differentiation of tumor-restraining myofibroblastic cancer-associated fibroblasts, challenging the existing notion that Treg cells enable tumor progression.

See related article by Zhang et al., p. 422 (5).

Within this framework comes the study in this issue by Zhang and colleagues that addresses the critical question of the interrelationship among the various stromal cells found in PDA and Treg cells (5). Using multiplex IHC of surgical tissue samples obtained from human PDA resections, the authors demonstrated that Treg cells are present in the majority of tumor samples and correlate with macrophage and CD8⁺ T-cell infiltration. Furthermore, they verified by immunostaining and single-cell sequencing analysis that Treg cells are present in pancreatic intraepithelial neoplasia (PanIN)-bearing KrasLSL/G12D, Ptf1a+/Cre (KC) as well as iKras⁺ and iKras⁺;p53⁺ mice. To understand the role of Treg cells in the formation of PanIN, they generated KrasLSL/G12D;Ptf1a+/Cre;Foxp3TGERTKR DTR/GFP;hY (KC;Foxp3TGERTKR) mice in which the human diphtheria toxin receptor (DTR) is expressed under the control of the Foxp3 locus. The most novel finding in this study is that the administration of diphtheria toxin to deplete Treg cells resulted in increased tumor burden. These observations together suggest that, in the presence of oncogenic Kras, depletion of Treg cells causes a deleterious inflammation that coorchestrates PanIN formation. Although these findings are ostensibly paradoxical to previous studies, Zhang and colleagues use an autocrine model of slowly progressing PDA. As such, the observations from this study can be compared to a limited extent to transplantation models that are much more aggressive and harbor a distinct TME.

Cancer-associated fibroblasts (CAF) represent a heterogeneous cell population. Among several other cell types, resident tissue fibroblasts are considered predecessors (6). In the pancreas, CAFs are prevalent in the stroma of PanIN lesions. They can deposit extensive amount of extracellular matrix, leading to a prominent desmoplastic reaction with a dense fibrotic stroma. The observations from this study can be compared to limited extent to transplantation models that are much more aggressive and harbor a distinct TME.

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¹S. Arthur Localio Laboratory and Department of Surgery, New York University School of Medicine, New York, New York. ²Department of Cell Biology, New York University School of Medicine, New York, New York.

Corresponding Author: George Miller, New York University School of Medicine, 435 East 30th Street, Room 417, New York, NY 10016. Phone: 646-501-2208; Fax: 212-263-6840; E-mail: george.miller@nyumc.org

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tumors, enhanced metastatic spread, and reduced survival (8). The current study goes beyond these previous findings and identifies Treg cells as a novel regulator of myCAFs. To investigate the extent to which myCAFs can induce immunologic reprogramming, the team analyzed the immune cell landscape of PanIN lesions after Treg cell depletion. They treated mice with caerulein and depleted Treg cells one week before sacrifice to allow for a similar severity of PanIN lesions. This treatment protocol allows the study of immune-cell infiltration independent of the PanIN lesion load. Importantly, despite mostly unchanged T-cell numbers, depletion of Treg cells increased F4/80+ macrophages/DCs with an inhibitory ARG1+ phenotype. Subsequent analysis of the secretome revealed that the influx of immune-suppressive myeloid cells is dependent on CCR1. To test whether the influx of immunosuppressive myeloid cells upon Treg cell depletion can be abrogated by pharmacologic blockade of CCR1, the authors used the CCR1 inhibitor BX471. They found that CCR1 blockade reduced dysplasia and fibrosis despite the depletion of Treg cells. Of note, CCR1 blockade was recently shown to have a synergistic antitumoral effect when used in combination with anti–PD-L1 in a murine model of breast cancer (9).

CD4+ T cells play a crucial role in antitumor immunity. Typically, they recognize peptides presented by MHC II molecules expressed on the surface of antigen-presenting cells such as macrophages or DCs. Such activation results in the differentiation of naive CD4+ T cells into T helper cells (Th) through activation of specific transcription factors. In pancreatic cancer, Th1-polarized CD4+ T cells are thought to mediate tumor protection. Conversely, Th2-polarized CD4+ T cells are associated with tumor-permissive anergy (1). Zhang and colleagues thus analyzed Th1 and Th2 subsets in pancreata of Treg cell–depleted mice. The team found that Treg cell–depleted KC;Foxp3<sup>Cre</sup> mice had increased concentrations of Th2-associated and decreased concentrations of Th1-associated cytokines. Finally, the authors showed that depletion of CD4+ T cells in KC;Foxp3<sup>Cre</sup> mice mitigated immune infiltration as well as ADM and resulted in decreased fibrosis (Fig. 1).

These findings of Zhang and colleagues add to an emerging theme that challenges the previous notion where Treg cells are seen as critical mediators of immune suppression in PDA. As such, previous work demonstrated that, despite the ostensibly tolerogenic Treg phenotype induced by PDA-infiltrating DCs, CD4+ T cells have negligible tumor-promoting or immune-suppressive functions when compared with Tr1 cells (10). Not only are these new insights important to the understanding of PDA, but the mechanisms uncovered in this study could have relevance for other tumor entities with a strong fibrotic component as well. Furthermore, could these discoveries shed light on the mystery of why so far in clinic, no Treg cell–targeted therapy has been proven to be effective? The information we learn from forthcoming studies investigating this question will allow us to not only understand the biology of Treg cells better but improve upon the current clinical outcomes as well.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**REFERENCES**

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