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Commentary

A Translational Perspective of a Deubiquitinase Inhibitor in Antitumor Immunity

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Foxp3$^+$ regulatory T (Treg) cells are known to restrict anti-tumor immune responses and promote tumor progression (Nishikawa and Sakaguchi, 2014). Therapeutic utility of attenuating Treg cell function is one of the most exciting developments in the current field of tumor immunology. Temporary release from the suppressive influence of Treg cells provides beneficial traits to support anti-tumor responses. However, most therapeutic trials can only partially deplete Treg cells or modulate Treg cell function indirectly (Pardoll, 2012). The work presented in this issue of *EBioMedicine* by Wang et al. (2016) provides a new immunotherapy strategy against tumors by targeting Usp7, which successfully dampens the immune suppressive function of Treg cells and limits tumor growth.

The immunosuppressive phenotype of Treg cells is largely determined by the gene expression patterns driven by Foxp3 (Fontenot et al., 2005). Ample evidence has supported that, in addition to transcriptional control of the *Foxp3* gene, the stability of Foxp3 expression is also determined at the post-translational level (Chen et al., 2013; van Loosdregt et al., 2013). Specifically, modification of Foxp3 protein by deubiquitination carried out by deubiquitinase Usp7 could stabilize Foxp3 protein, effectively strengthening Treg cell function (van Loosdregt et al., 2013). Meanwhile, the histone acetyltransferase Tip60 also plays a dominant role in promoting acetylation, dimerization and function of Foxp3 in Treg cells (Li et al., 2007; Xiao et al., 2014). Therefore, these pathways of post-translational Foxp3 regulation significantly impact the *in vitro* and *in vivo* function of Treg cells.

The work by Liqing Wang et al. starts with genetic evidence that developmental deletion of Usp7 in Treg cells induces lethal systemic autoimmunity. Usp7 depletion perturbs Treg signature gene expression, including the Foxp3 co-regulators Eos and Gata1, and dampens the suppressive activity of Treg cells. Usp7$^{-/-}$ Treg cells also acquire the expression of glycolytic genes and proliferate actively. More interestingly, Usp7$^{-/-}$ Treg cells express more IFN-γ and display a Th1-like phenotype. All these data suggest that inhibition of Usp7 might impair Treg cell function and potentially promote anti-tumor immunity. Therefore, pharmacologic blockade of Usp7 might provide therapeutic clues towards the effective immunotherapy against cancer.

Interestingly, Treg cells abrogated their suppressive activity after pre-treatment with Usp7 inhibitor (Usp7i) P5091 or its derivative P0217564, which is accompanied by dramatic loss of Tip60. These observations were consistent with previous studies that Tip60 critically regulates the function of Treg cells through promoting acetylation, dimerization and function of Foxp3. Meanwhile, Usp7i treatment promoted the ubiquitination of both Foxp3 and Tip60, and also inhibited the formation of Foxp3 dimers. However, the expression level of Foxp3 protein was comparable in WT and Usp7$^{-/-}$ Treg cells, which suggests the existence of a redundant role of Usp7 in stabilizing Foxp3 protein. Therefore, pharmacologic inhibition of Usp7 impairs Treg cell function, likely through mechanisms that are Tip60-dependent rather than simply *via* Foxp3 ubiquitination.

The therapeutic application of Usp7i in several mouse tumor models shows promise, especially the combination of Usp7i with other antitumor therapies. Usp7i treatment decreased the intratumoral accumulation of Foxp3$^+$ Treg cells and significantly inhibited tumor growth. More importantly, Usp7i treatment promoted the accumulation of IFN-γ-producing tumor-antigen specific CD8$^+$ T cells in the tumor microenvironment, which greatly contributed to the temporal release of immune tolerance. Therefore, from a translational perspective, Usp7i-mediated Treg cell inhibition shows potential as a novel approach in the immunotherapy against tumor.

The fact that Usp7 has multiple substrates could be one of the major concerns in clinical trials of Usp7 inhibitors (Nicholson and Suresh Kumar, 2011). Another problem could be the wide expression of Usp7 in many cell types. Therefore, Usp7 inhibitors might affect other cell populations rather than simply inhibit the function of Treg cells through Tip60 *in vivo*. Another preclinical study showed that Usp7i directly induced apoptosis in multiple myeloma cells (Chauhan et al., 2012). However, from the proposed molecular mechanism in the current study by Wang et al., Usp7i compounds can have dominant effects on Foxp3$^+$ Tregs over that of other immune cell types, including host effector T cells and CD8$^+$ T cells. Therefore, pharmacologic modulation of Treg

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cells using Usp7i compounds gives a new potential to break the immune tolerance in the tumor microenvironment.

In summary, the data by Wang and colleagues proposes a potential immunotherapy against tumors by targeting Usp7, which impairs Treg cell function and subsequently breaks the immune tolerance in the tumor microenvironment. Therefore, these preclinical findings suggest that Usp7 targeting immunotherapy to selectively diminish Treg cell function, as well as to directly induce tumor cell apoptosis, could have practical significance in clinical applications.

Disclosures

The authors declare no conflicts of interest.

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