



## RESEARCH HIGHLIGHT

# Immune regulation by CD8<sup>+</sup> Treg cells: novel possibilities for anticancer immunotherapy

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Regulatory T (Treg) cells comprise diverse subsets of immunosuppressive cells that play a pivotal role in regulating immune homeostasis and preventing autoimmunity. In cancers, Treg cells can suppress antitumor immune responses and support the establishment of an immunosuppressive tumor micro-environment, thus promoting immune evasion and cancer progression.<sup>1</sup> Treg cells are increased or activated in the tumor microenvironment, which is associated with a poor clinical outcome.<sup>2</sup> Although CD4<sup>+</sup> Treg cells have been extensively studied, the lack of universal markers to distinguish CD8<sup>+</sup> Treg cells from conventional CD8<sup>+</sup> T cells means that the function of CD8<sup>+</sup> Treg cells in cancer has not been fully characterized. Now, an increasing body of research has revealed that CD8<sup>+</sup> Treg cells (CD8<sup>+</sup> CD25<sup>+</sup> Foxp3<sup>+</sup>, CD25<sup>+</sup>CD122<sup>+</sup>Foxp3<sup>+</sup> and CD8<sup>+</sup>CD28<sup>-</sup>)<sup>3–5</sup> accumulate in the tumor microenvironment and suppress antitumor immunity (Fig. 1). However, the influence of CD8<sup>+</sup> Treg cells on tumor progression in ovarian cancer (OC) is less clear. Moreover, there are a limited number of studies describing the molecular signatures involved in the induction of CD8<sup>+</sup> Treg cells.

We recently identified the expression of Treg markers in CD8<sup>+</sup> T cells isolated from peripheral blood and fresh tumor tissues of OC patients. We detected a higher percentage of CD8<sup>+</sup> Treg cells in OC patients compared with benign ovarian tumor patients and healthy controls.<sup>6</sup> The immune-suppressive T-cell markers CD25, cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and Foxp3 were upregulated, whereas expression of the immune activation marker CD28 was downregulated in these cells. We also showed that levels of Foxp3 in CD8<sup>+</sup> T cells were positively associated with tumor stage in OC patients, suggesting that CD8<sup>+</sup>Foxp3<sup>+</sup> Treg cells contribute to the progression of OC. This highlights the role of CD8<sup>+</sup>Foxp3<sup>+</sup> Treg cells as predictors of clinical outcome in OC patients. Then we determined whether the tumor microenvironment had the ability to convert CD8<sup>+</sup> effector T cells into suppressor cells. We observed phenotypic similarities between CD8<sup>+</sup> T cells in transwell co-culture system and CD8<sup>+</sup> T cells obtained from OC tumor tissues, including upregulated Foxp3 and CTLA-4 and downregulated CD28 expression levels.<sup>6</sup> The in vitro-induced CD8<sup>+</sup> Treg cells also secreted higher concentrations of transforming growth factor β1 (TGF-β1), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2. The Foxp3<sup>+</sup>CTLA-4<sup>+</sup> and TGF-β1<sup>+</sup> phenotype indicated that the induced CD8<sup>+</sup> Treg

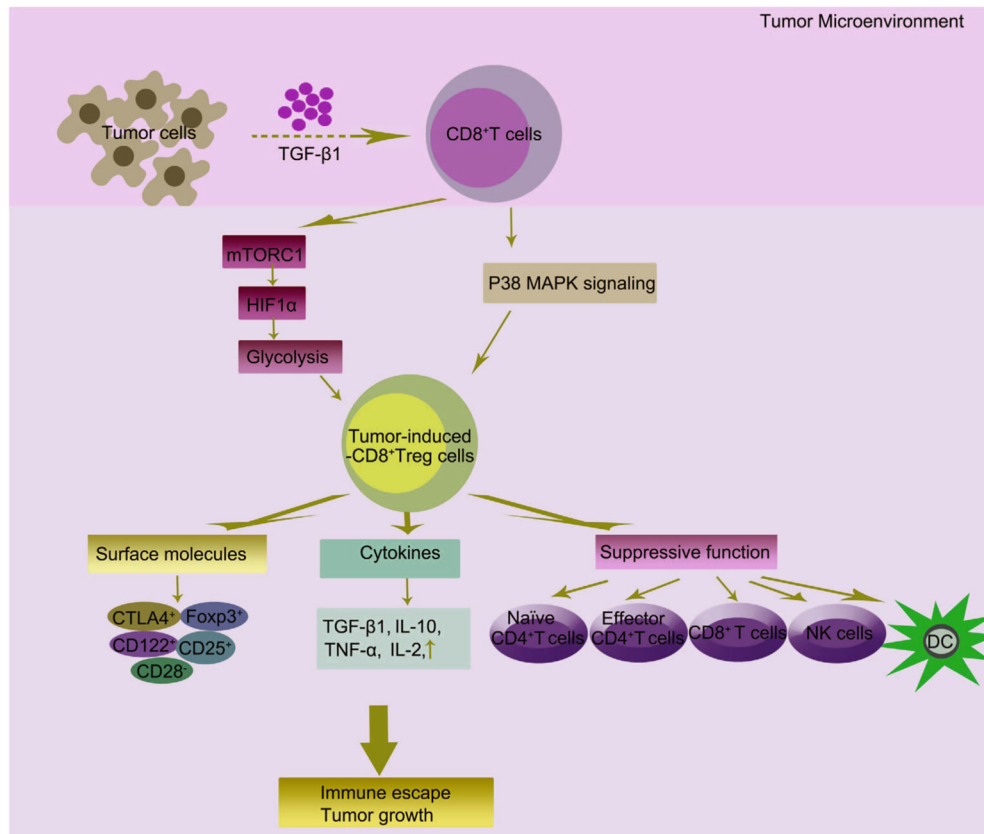
cells were functionally activated, which was confirmed by their ability to suppress CD4<sup>+</sup> T-cell proliferation partially by TGF-β1 and IFN-γ. These data imply that CD8<sup>+</sup> Treg cells accumulated in OC tissue in vivo and inducible in vitro may dampen antitumor immunity and contribute to tumor immune evasion.

TGF-β1 also has a crucial role in the development of Treg cells,<sup>7</sup> and CD4<sup>+</sup> CD25<sup>-</sup> T cells deficient in the TGF-β signaling pathway cannot be converted into Foxp3<sup>+</sup> iTreg cells.<sup>8</sup> We showed that OC SKOV3 cells induced CD8<sup>+</sup> Treg cells by secreting TGF-β1 and that OC patients expressed high levels of TGF-β1, correspondingly, increased TGFβ1 secretion was demonstrated in supernatant from the co-culture system of CD8<sup>+</sup> T cells and SKOV3. Additionally, TGF-β1 levels were positively correlated with the percentage of CD8<sup>+</sup> Treg cells in OC.<sup>9</sup> Consistent with previous reports,<sup>10</sup> we also confirmed that high expression TGF-β1 at least partially contributed to the suppressive function of in vitro-induced CD8<sup>+</sup> Treg cells. Compared with CD8<sup>+</sup> T cells cultured alone, CD8<sup>+</sup> T cells co-cultured with SKOV3 cells exhibited marked activation of p38 mitogen-activated protein kinase (MAPK), which could be inhibited by a TGF-β1-neutralizing antibody. Moreover, the TGF-β1-triggered conversion to CD8<sup>+</sup> Treg cells in the tumor microenvironment CD8<sup>+</sup> Treg cells in vitro was dose-dependently blocked by the p38-specific inhibitor SB203580, indicating that the induction of CD8<sup>+</sup> Treg cells depended in part on TGF-β1-activated p38 MAPK signaling. These findings indicate that TGF-β1 is a potential target in the treatment of OC.

We further investigated the molecular signatures that contributed to the induction of CD8<sup>+</sup> Treg cells by comparing the expressed gene profiles when CD8<sup>+</sup> T cells were cultured with or without SKOV3 cells.<sup>11</sup> DNA microarray data showed that 73% of previously reported CD8<sup>+</sup> Treg cell molecular markers were significantly upregulated during co-culture with SKOV3 cells. Among them, ITGAX, also known as CD11c, was particularly noteworthy. This observation is consistent with previous reports that CD11c<sup>high</sup>CD8<sup>+</sup> Treg cells possess potent cytotoxicity to target cells via the Fas/Fas ligand pathway and that this subset of CD8<sup>+</sup> Treg cells can inhibit the CD4<sup>+</sup> T-cell-mediated immune response by killing the activated CD4<sup>+</sup> T cells.<sup>12</sup> Interestingly, we found that the expression of glycolysis genes in CD8<sup>+</sup> T cells was decreased during co-culture with SKOV3 cells, while their expression was negatively correlated with Foxp3 expression in CD8<sup>+</sup> Treg cells. This indicated that the glycolysis pathway may contribute to the differentiation and generation of CD8<sup>+</sup> Treg cells. Further efforts are needed to fully reveal the underlying

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**Fig. 1** Tumor-induced CD8<sup>+</sup> Treg cells and their potential role in tumor microenvironment. TGF-β1 can be released by cancer cells in the tumor microenvironment. The suppressive activity of Treg cells mainly mediated through tumor-derived exosome TGF-β1. We previously reported that TGF-β1 secreted by OC cells could generate CD8<sup>+</sup> Treg cells by promotion of the p38 MAPK signaling pathway. Tumor microenvironment influences T-cell immune responses by altering cellular metabolism. mTORC1 regulates glucose metabolism in CD8<sup>+</sup> Treg cells' differentiation through regulating the expression of HIF1α. Treg cells use different strategies to inhibit target cells within the tumor mass. Among the surface molecules expressed by CD8<sup>+</sup> Treg cells, CD25, CD122, CD28, CTLA-4 and Foxp3 have a well-demonstrated role in promoting tumor progression. Treg cells secrete several immune-modulatory cytokines (TGF-β, IL-10, TNF-α and IL-2). Tumor-induced-CD8<sup>+</sup> Treg cells could also directly modulate activation and function of immune cells. CD8<sup>+</sup> Treg cells may play a role in promoting imbalance of the immune system in tumor growth. These immune-tolerance mechanisms may also be exploited by cancer cells to achieve immune escape, which becomes more pronounced with tumor formation, progression and metastasis. CTLA-4, cytotoxic T-lymphocyte associated protein 4; HIF1α, hypoxia-inducible factor 1α; IL, interleukin; MAPK, mitogen-activated protein kinase; mTORC1, mammalian target of rapamycin complex 1; OC, ovarian cancer; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; Treg, regulatory T

mechanism of CD8<sup>+</sup> Treg cells' differentiation and activation in an ovarian microenvironment.

Taken together, these findings suggest that CD8<sup>+</sup> Treg cells accumulate in human OC tissues and display an activated phenotype with suppressive functions. Although CD8<sup>+</sup> Treg cells represent only a small fraction of CD8<sup>+</sup> T cells in vivo, targeting CD8<sup>+</sup> Treg cells may potentiate the antitumor immunity and rebalance the OC microenvironment. We are attempting to confirm the clinical relevance of this hypothesis.

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#### ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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