

Targets specific to certain Treg cell types in the TME of pancreatic cancer

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Abstract. The increased content of Treg cells in the pancreatic cancer TME shows the development of pancreatic cancer is largely influenced by treg cells. Regulatory T cells (Treg) have a powerful immune suppression function, under normal circumstances, have the function of maintaining autoimmune tolerance. When Treg cells expressing abnormally in tumor tissue, they can inhibit normal anti-tumor immune response, accelerate the immune escape in tumor cells, promote tumor growth. Meanwhile, Treg can also inhibit tumor growth through anti-inflammatory and anti-angiogenesis routes. Blind killing and inhibition of Treg will destroy the maintenance of the body's self-immune tolerance and cause some damage. In this paper, the duality of Treg cells is summarized through two aspects, including the summary analysis of specific targets on different types of Treg cells in TME to find the targets that can specifically kill immunosuppressive Treg cells; discuss the interactions between Treg cells and their surrounding cells, and their relationship with the duality of Treg cells, in order to provide the basis for reducing damage and optimizing immunotherapy during immunotherapy.

Keywords: Pancreatic cancer; Treg; immunosuppression; Antitumor property; Specific target.

1. Introduction

Pancreatic cancer is among the deadliest human malignancies, with a 5-year survival rate of approximately 3% and 11% at all stages, the relatively lowest of all malignancies [1]. Pancreatic cancer is usually diagnosed in the advanced stage and has the characteristics of difficult to diagnose and treat. In clinical practice, tumor immunotherapy has been used, and a great deal of attention has been given to the role of TME in tumor immunity. TME is made up of malignant cells (tumor cells), normal cells and cytokines (figure 1), normal cells including lymphocytes, bone marrow cells, macrophages, neutrophils, fibroblasts and formation of blood formation, these cells can secrete soluble factors, through soluble signal molecules, such as attracting immune cells, induce immune cell reorganization, in promoting, inhibit the development of tumor plays a key role [2]. It has been discovered that Treg cells in the TME are crucial in the progression of pancreatic cancer. In general, Treg cells can limit inflammation, ensure the body's immune homeostasis is in check, and avoid the occurrence of self-immunity. When Treg cells are abnormally expressed in the TME, it can also promote the tolerance of peripheral tumors and exert their immunosuppressive effect [3].

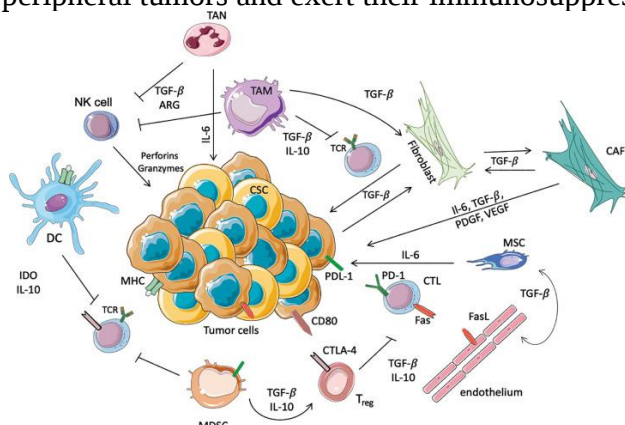


Fig. 1 Composition of the tumor-immune microenvironment [2]

In 2014, Zhang et al. found that a large accumulation of Treg cells was visible in mouse and human precursor pancreatic intraepithelial neoplasia (PanIN) and pancreatic cancer, which speculated that Treg cells have important significance in promoting the advancement of pancreatic cancer [4]. It is evident that Treg cells play a negative role in the TME of pancreatic cancer, which can promote the occurrence and evolution of tumors by hindering the body's immune response. Based on the immunosuppressive nature of Treg, in 2020, Zhang et al. wanted to use diphtheria toxin (DT) to eliminate their immunosuppressive effect and inhibit tumor growth but found that the genesis and incidence of pancreatic cancer had promoted [5]. This shows that Treg can be anti-tumor under certain conditions. This goes against the immunosuppressive properties of Treg cells, showing that Treg cells have dual properties, namely, immunosuppressive, and anticancer properties. In addition, Xu et al. performed ligand-receptor analysis and found strong cell-cell interactions between M2 macrophages, Treg cells and X, CD8⁺ TEX cells through chemically directed, immunosuppressive or costimulatory factors [6]. Therefore, Treg can play their immunosuppressive effects indirectly by acting on NK cells. It may therefore be considered whether the immunosuppression and antitumor effects of Treg cells are achieved by interacting with the other cells in TME. This effect can be directly with immune cells or with cells that do not have immune effects.

Although the above studies found that Treg cells have duality, how the duality is reflected, whether there are two Treg cells with different functions or different types of targets on the same Treg cells have different effects due to the different activated targets, and the specific mechanism is not clear. At present, there are many targeted therapeutic drugs for Treg cells, but their targets are not specific. Because the targets are widely found on various types of Treg, the killing inhibition of Treg is universal, so it often brings adverse effects on patients. In this paper, we discuss the duality of Treg cells in terms of the two aspects of Treg cells themselves and the interaction between Treg and its surrounding cells, so as to provide a theoretical basis for the more accurate targeted therapy of Treg cells to reduce the adverse effects.

2. Treg cell heterogeneity

In the process of tumor development, Treg cells play an extremely important role in TME. Treg cells can promote the development of tumors by inhibiting the immune function of the body. Meanwhile, Treg cells can also inhibit the development of tumors. According to its biological characteristics, there are two types of Treg.

Treg cells with anti-tumor properties are natural regulatory T cells (naturally occurring Tregs, nTregs), and these Treg cells can inhibit the development of tumors through anti-inflammatory, anti-autoimmune, anti-angiogenesis and other channels. After the depletion of Treg cells in pancreatic cancer mice with diphtheria toxin (DT), the ratio of the weight of the spleen to the total weight of the mice increased, suggesting that the mice developed inflammation [5]. The anti-inflammatory effect of Treg cells was seen.

The Treg cells with immunosuppressive properties are induced regulatory T cells (induced Tregs, iTregs). The main function of these Treg cells is to inhibit the body's immune response and maintain immune tolerance. The main mechanisms are: through the secretion of immunosuppressive factors, including: ① IL-10 and TGF- β promote differentiation of peripheral T cells into Treg, ② IL-2, IL-15, and IL-35 promote the proliferation of Treg cells and activate, ③ PD-L1 promotes the activation of Treg cells by binding to PD-1 on Treg cells, ④ IDO and PEG2 inhibit the killing effect of immune cells on Treg cells, ⑤ VEGF: promotion of tumor angiogenesis, promote tumor metastasis; Immunosuppressive Treg cells can also be in direct contact with immune cells, promote their phagocytosis, reduce the number of immune cells; At the same time, Treg cells have metabolic competition with immune cells, can inhibit their growth (Figure 2). Precise classification of two Treg cells with antagonism in tumor immunotherapy and targeted killing of immunosuppressive Treg cells increased the proportion of antitumor Treg cells and reduced the adverse effects on patients.

Searching for specific targets of two types of Treg cells allows a more precise distinction of Treg cells.

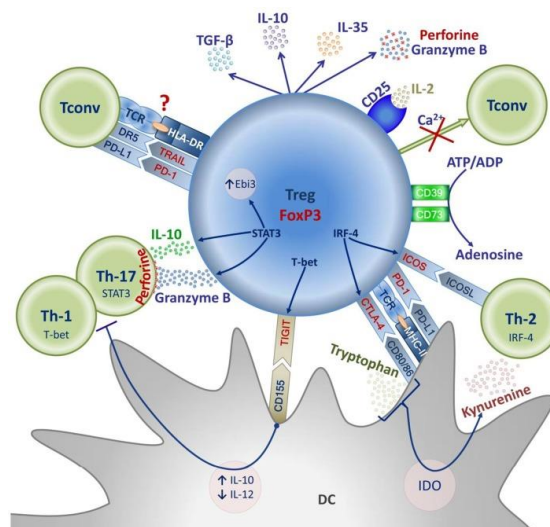


Fig. 2 Immunosuppressive effects of Treg cells [7]

3. Specific targets of Treg cells

3.1. Specific targets of antitumor Treg cells

Natural regulatory T cells (naturally occurring Tregs, nTregs), also known as thymic regulatory T cells (tTregs), originate from the thymus and function to maintain a normal immune tolerance and manage inflammation.

Further studies of the pathway, molecular mechanism, and signaling pathways that produce granzyme B can be used as one of the specific targets to distinguish nTreg from iTreg. In nTreg, some transcription factors, including signal, were observed to have DNA hypomethylation at the Foxp3 CNS2 site, but not in pTreg [8]. It can be inferred that the translation proteins, molecules, and signaling pathways involved in the expression of FOXP3 are different between nTreg cells and iTreg cells, and further differentiation can be used as specific targets for targeting Treg cells.

3.2. Specific targets of immunosuppressive Treg cells

Inducible Tregs (iTregs), also known as peripheral Tregs (pTregs), are evolved from peripheral naive CD4⁺ T cells and consist of tumor antigens, cytokines (e. g., TGF-β), etc. Mainly involved in the immune escape of the forming tumor cells.

The targets that can specifically target iTreg cells and the related targeted drugs as is shown in table 1.

Table 1. Summary of the specific target and the targeted medicine [9]

Medicine	Target
LY3475070	Ectonucleotidase CD73
ADCT-301	CD25 (ADC conjugated)
Denileukin diftitox	CD25(toxin conjugated)
R07296682	CD25 (without IL-2 signal blockade)
TIX-030	Ectonucleotidase CD39
Ipilimumab	CTLA-4
PBF-509	Adenosine receptor A2A
Kinase inhibitors	VEGFR2
IOA-244	PI3K8
Mogamulizumab	CCR4
MEDI6469	OX40
TRX518	GITR
JTX-2011	ICOS
VT1021	CD36
AZD3965	MCT1
Epacadostat	IDO

4. Interaction of Treg cells with other cells in the TME

4.1. Interactions between Treg cells and fibroblasts

Under normal conditions, Treg cells regulate the secretion of TGF- β and promote the differentiation on fibroblasts into SMA-expressing cancer-associated fibroblasts, which inhibits the development of tumors. After the depletion in Treg cells, the production on TGF- β is reduced, and the differentiation of SMA + fibroblasts is reduced. At the same time, several CCR1 ligands on the surface of fibroblasts secrete a large amount of bone marrow to recruit chemokines, recruit a large number of bone marrow cells, produce immunosuppressive effects on the body, and enhance the immunosuppression (Figure 3) [10]. Therefore, CCR 1 can be used as a specific target for regulating Treg cells, thereby inhibiting the immunosuppressive effect of fibroblasts, and thus playing the role of indirect inhibition of immunosuppressive Treg cells.

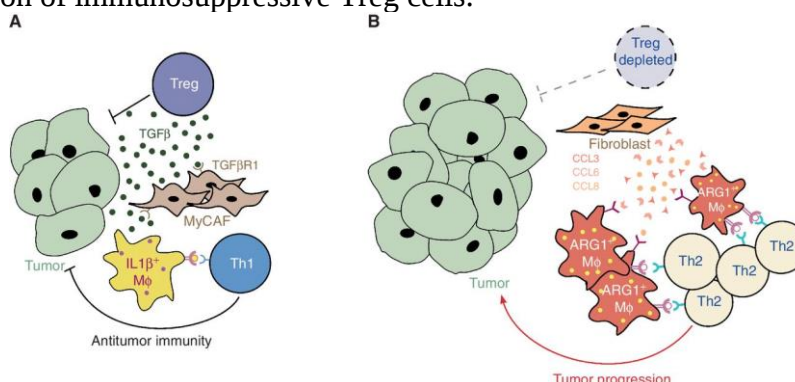


Fig. 3 Mechanisms of the interaction between Treg cells and fibroblasts [10]

4.2. Interactions between Treg cells and MDSC cells

Following the disappearance with Treg cells, the increase in MDSC cells in TME promotes the development of pancreatic cancer through compensatory immunosuppression, but the specific mechanism is currently unknown [5]. The Treg cells are known to have anti-inflammatory effects. When the Treg cells are depleted, the body can produce inflammation. Therefore, it can be speculated that the mechanism of compensatory immune suppression is: the mediators produced by inflammation (such as IL-1 β , IL-6, VEGF) to further enhance the interplay between these two cells,

thereby enhancing the secretion of IL-10 and TGF- β or promoting STAT-3 signaling, contribute to cell surface molecules that participation in bidirectional positive feedback loops (e. g. PD-L1) and the enzyme (CD39, Upregulation of either Nox-2 or Arg-1). Then further promotes the proliferation and differentiation of MDSC, which mediates the immunosuppressive effects.

4.3. Interactions between Treg and CD4 + T

After Treg cell depletion, a pathological CD4 + T cell response can promote the development of pancreatic cancer, but the specific mechanism is currently unclear (Figure 4) [5]. Treg has an interaction with Th17 and Th2. When Treg cells are depleted and the content of pathogenic Th2 and Th17 increases, which promotes the development of pancreatic cancer, Th2 and Th17 can be used as indirect targets of immunotherapy against Treg cells, thus reducing the immunosuppressive effect and indirectly inhibiting the immunosuppressive effect of Treg cells.

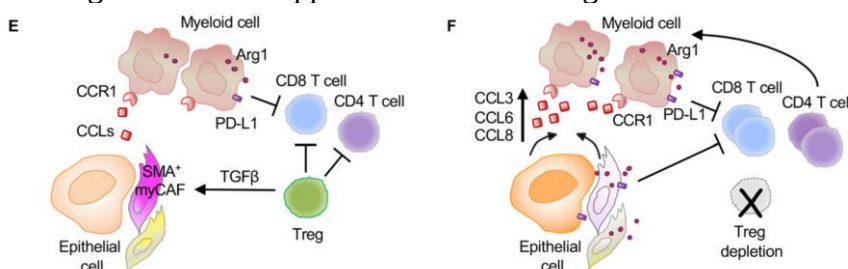


Fig. 4 The interaction between Treg and CD4 + T cell [5]

5. Summary

Treg cells are of two types, tTreg is natural Treg cells from the thymus, which mainly maintain immune tolerance and anti-inflammatory, and pTreg cells are induced Treg cells from the periphery, which mainly suppress immunity. It is of great significance to find the unique targets of the two cell classes for specific targeted therapy for cells. In this paper, the specific targets of the two Treg types were distinguished to provide specific therapeutic targets for specifically inhibiting killing immunosuppressive Treg cells, maintaining the level of anti-tumor Treg cells, and maintaining the number of normal Treg cells while improving the immune function of the body.

Most of the existing studies on Treg cell targets are non-specific. When immunotherapy on Treg cells is conducted, it will inhibit and kill immunosuppressive Treg cells and anti-tumor Treg cells, leading to the destruction of the body's own tolerance and causing damage to the body. Differentiating Treg cells precisely prevents blind destruction of Treg cells and results in the loss of normal Treg cells. In this paper, distinguishing between the targets unique to the two types of Treg cells can provide precise targets for immunotherapy in the clinic. At the same time, there is an interaction between Treg and other immunosuppressive cells, so the specific targets of them can be explored to exert the killing inhibitory effect on immunosuppressive Treg cells through the effects on these cells.

Based on the basis of the specific targets of different cell types, tissue-specific differentiation can be made. That means finding the unique targets on Treg cells in the TME in a specific tissue, which can only kill Treg in a specific tissue during immunotherapy and has no effect on other normal tissues.

In the future, we can also explore whether there are different targets on the same Treg cells, and when activating these different targets, the same Treg cells have different or even antagonistic effects.

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