

Research Progress of OX40/OX40L in Tumor Immunotherapy

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Abstract: T cell co-stimulatory receptor OX40 (CD134) and its ligand OX40L (CD252) are members of the tumor necrosis factor receptor/tumor necrosis factor superfamily (TNFRSF/TNFSF), respectively. OX40 is mainly expressed on activated T cells, including CD4, CD8, helper T cells, and regulatory T cells (Tregs). OX40L is mainly expressed on antigen-presenting cells (APCs). OX40/OX40L are a pair of co-stimulatory molecules that play vital roles in both initial and secondary T-cell responses. They are critical for the maintenance of T-cell proliferation, survival, and the formation of memory T-cells, which affects cellular immunity, humoral immune response, and immune tolerance. It is pivotal in mediating the occurrence and development of tumor immune response. Preclinical animal studies have demonstrated that OX40-targeted agonists can exert significant anti-tumor effects when used alone or in combination with other treatment methods. Multiple early clinical studies targeting OX40/OX40L are currently underway. This article will review molecular biological characteristics, mechanisms of action, and anti-tumor applications.

1. Introduction

With the continuous deepening of research on tumor immunotherapy, the efficacy of cytotoxic T lymphocyte associated antigen-4 (CTLA-4) antibody and programmed death 1/programmed death ligand 1 (PD-1/PD-L1) antibody has become more and more prominent in clinical treatments, which further confirms the key role of the immune system in tumor therapy. However, with the emergence of resistance problems with first-generation immune checkpoint inhibitors, there is an urgent clinical requirement for new immunomodulatory antibodies that break tumor cell-mediated immune tolerance through multiple signaling pathways to overcome drug resistance. Currently, research is focused on antigen presentation and INF- γ signaling pathway, the combination therapy of next-generation immune checkpoints has broad development prospects in overcoming drug resistance issues. The immune costimulatory molecule OX40/OX40L stands out and has become a new target for next-

generation tumor immunotherapy.

2. Biological Characteristics of OX40 and OX40L

OX40 also called as CD134, ACT45, TNFRSF4, is a type I transmembrane glycoprotein that belongs to the tumor necrosis factor receptor (TNFR) superfamily. It is composed of three components: intracellular, transmembrane and extracellular. It is an activation receptor expressed on the surface of activated CD4⁺Th (Th1, Th2, Th17, etc.), CD8⁺T cells, and Foxp3⁺CD25⁺ regulatory T cells (Treg). OX40L, also called as CD252, is a type II transmembrane glycoprotein and the only ligand for OX40. It is mainly expressed on the surface of activated antigen presenting cells (APCs), such as B cells, mature dendritic cells (DCs), Langerhans cells, megaphages, etc. It is also expressed on the surface of natural killer cells and mast cells. In addition, when inflammatory cytokines are present in the microenvironment, they are also expressed on the surface of smooth muscle or endothelial cells[1]. The OX40/OX40L signal can activate downstream NF- κ B, PI3K and PKB pathways. The sustained activation of these pathways can ultimately prolong the survival time of T cells, expand T cell memory, and promote T cell cytotoxicity. In addition, OX40 can improve the immunosuppressive effect in the tumor microenvironment by inhibiting the differentiation and activity of Treg cells, further enhancing the function of effector T cells.

3. The Function of OX40/OX40L in Cellular Immunity

T cells are one of the main cells that mediate adaptive immune responses. The activation of initial T cells requires dual signals: APC presenting MHC antigen peptide complexes that bind to TCR-CD3 of T cells and provides the first signal for T cell activation; T cell surface receptor molecules, represented by CD28, bind to the corresponding ligand B7 to transmit a synergistic stimulus signal, which is the second signal of T cell activation. OX40 is a co-stimulatory molecule on the surface of T cells, which binds to OX40L on the surface of APC and participates in the transmission of the second signal of T cell activation. The activation of the OX40/OX40L costimulation pathway is an important condition for T cell differentiation, proliferation, survival and migration. Its research in the field of tumor immunotherapy is constantly deepening.

3.1. OX40/OX40L and CD4⁺Th Cells

The OX40/OX40L co-stimulatory pathway can promote the proliferation, survival, and migration of CD4⁺Th cells. Research has shown that this effect is achieved by enhancing the survival ability of CD4⁺Th cells, rather than by increasing the rate of cell proliferation. CD4⁺Th cells with OX40 gene defects cannot maintain differentiation after recognizing antigens, and memory cell generation is impaired. They also fail to produce sufficient anti apoptotic proteins, leading to apoptosis[2]. The mechanism was initially believed to be that the OX40/OX40L co-stimulatory pathway activated the PKB/AKT pathway, upregulating the anti-apoptotic protein "survivin" to promote CD4⁺Th cell survival. However, through retroviral transduction, the survivin gene was overexpressed in CD4⁺Th cells with OX40 gene deficiency, which could not achieve long-term survival, suggesting that its mechanism should be non survivin dependent. The mechanism was initially believed to be that the OX40/OX40L co-stimulatory pathway activated the PKB/AKT pathway, upregulating the anti-apoptotic protein "survivin" to promote CD4⁺Th cell survival. However, through retroviral transduction, the survivin gene was overexpressed in CD4⁺Th cells with OX40 gene deficiency, which could not achieve long-term survival, suggesting that its mechanism should be non survivin dependent. In terms of migration, the OX40/OX40L co-stimulatory pathway can upregulate the expression of chemokines in CD4⁺Th cells, promoting their entry into the bloodstream and migration

to the tumor site. Blockade of the OX40/OX40L co-stimulatory pathway can prevent CD4⁺Th cells from infiltrating the tumor site, indicating that the OX40/OX40L co-stimulatory pathway is critical for the migration of effector Th cells [3].

3.2. OX40/OX40L and CD8⁺T Cells

OX40 can promote the generation of CD8⁺T memory T cells and enhance the re-immune response to tumor associated antigens. By tracking the production of CD8⁺T cells in a mouse model infected with acute influenza virus, it was found that OX40 had little effect on the initial immune response of CD8⁺T cells. However, the number of tumor related CD8⁺T memory T cells in lymphoid and non lymphoid tissues of OX40L deficient mice decreased compared to normal mice. When encountering antigen stimulation again, the number of memory T cells that exert effects in their bodies also decreased compared to normal mice. However, research on mouse models of cowpox virus infection found that the number of CD8⁺memory T cells produced in OX40 deficient mice was not only reduced compared to normal mice, but their initial immune response was also significantly weakened. The effect of OX40 on CD8⁺T cells varies for infections with different pathogens, and it is currently speculated that it is mainly related to the different types and quantities of cytokines (IFN-I and IL-12) produced during the immune response process, which are closely related to the generation of CD8⁺T cells.

3.3. OX40/OX40L and Treg Cells

Regulating T cells is a group of lymphocytes that negatively regulate the immune response, marked by the transcription factor Foxp3 as a molecular marker, playing a crucial role in maintaining self-tolerance and avoiding excessive immune response damage. The regulation of T cells is mainly divided into two categories: naturally regulated T cells (nTregs) and adaptive regulated T cells (iTregs). The effect of the OX40/OX40L co-stimulatory pathway on Treg is not yet fully understood. Research has shown that OX40/OX40L plays an important role in the production of Treg, and OX40 has a strong antagonistic effect on TGF- β . Affects the conversion of natural T cells to Foxp3⁺Treg, thereby affecting the production of Treg. The total amount of peripheral Treg in mice with OX40 gene knockout is similar to that of normal mice, and their inhibitory ability on effector T cells is similar in vitro testing. However, the inhibitory effect of OX40 gene deficient Treg on effector T cells in vivo is much weaker than that of normal Treg cells, and its migration ability is greatly reduced [4]. It can be seen that the activation of the OX40/OX40L co-stimulatory pathway can promote Treg amplification, but the inhibitory effect of amplified Treg on effector T cells is weak, and PD-1 is upregulated in cells, causing these Treg cells to enter a state of exhaustion and possibly further apoptosis. This process can be blocked by exogenous IL-2, and if given the correct cytokine stimulation, these proliferating Tregs can have strong activity. The interaction between TGF- β , IL-2 and some cytokines such as vitamin A can enable peripheral initial T cells to obtain Foxp3 labeling, thereby becoming iTreg. When OX40 is not expressed, the number of T cells converted to iTreg increases. Conversely, when OX40 agonist antibodies are administered, CD4⁺T cells can be significantly inhibited from converting to iTreg. Its mechanism may be that OX40 directly intervenes in TGF- β R signal transduction, combined with OX40 mediated cytokine production by T cells and APC generated cytokine feedback inhibition of Foxp3, thereby inhibiting iTreg generation.

The degree of activation of OX40/OX40L signaling depends on the inflammatory microenvironment in which T cells are located. If there is a lack of IFN- γ and IL-4, OX40/OX40L can actually promote the proliferation of Treg. In summary, the effect of OX40/OX40L on Treg depends on its tumor microenvironment.

4. Signal Transduction of OX40/OX40L

The interaction between OX40 and OX40L can recruit TNFR related molecules (TRAFs), forming signal transduction complexes molecules containing IKK α , IKK β , PI3k and PKB (Akt). OX40 also synergizes with TCR signaling, enhancing intracellular Ca²⁺ through unknown mechanisms, thereby enhancing NFAT entry into the nucleus. OX40 can activate classic NF- κ B1 pathway or non-classical NF- κ B2 pathway, PI3k/PKB and NFAT pathway. Further upregulation of genes that control T cell division and survival, as well as promotion of transcription of cytokine genes and expression of cytokine receptors, is crucial for cell survival[5]. OX40/OX40L signaling can also cause downregulation of CTLA-4 and Foxp3. (Figure 1)

The function of OX40/OX40L mainly relies on two signaling pathways: an independent non antigen dependent signaling pathway and an antigen driven TCR signaling pathway [6]. OX40 molecules interact with TNFR related factors (TNAF2, 3, 5), the subunit p85 of I κ B kinase (IKK α , IKK β , IKK λ) intracellular phosphatidylinositol kinase and protein kinase B in the form of trimers, they form a complex together. This signal transducer leads to phosphorylation and degradation of the NF- κ B1 suppressor I κ B α , which in turn leads to activation of NF- κ B1 and entry of p50 and RelA into the nucleus, thus providing a signal for T cell survival in the absence of antigenic stimulation. When NF- κ B induced kinase recruitment to the complex will lead to NF- κ B2 is activated and can also provide a signal for T cell survival. When stimulated by antigens, the signal transducer of OX40, in conjunction with TCR recruited phosphoinositol dependent protein kinase 1, promotes Akt phosphorylation. The TCR signal initiates intracellular calcium influx, leading to dephosphorylation and entry into the nucleus of NFAT. OX40 increases intracellular calcium ion influx and accumulation of NFAT in the nucleus. Furthermore, the TCR signaling and OX40 signaling pathways can upregulate the expression of survivin, auroral protein, cyclin A, cyclin dependent protein kinase, anti-apoptotic molecules (such as Bfl-1, BCl-2, and BCl-xL), as well as some cytokines and receptors, and downregulate the expression of Foxp3 and toxic T cell related antigens.

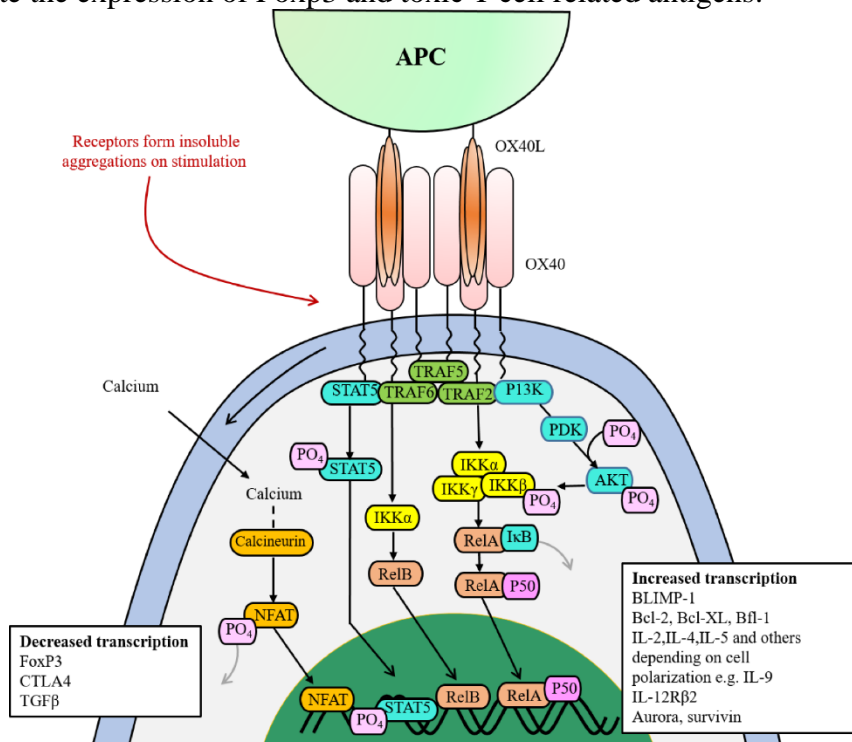


Figure 1: Signal transduction pathways after OX40/OX40L activation.

5. OX40/OX40L and Tumor Immunotherapy

OX40 is expressed on the surface of tumor infiltrating lymphocytes (TILs) in a variety of different tumor, including liver cancer, gastric cancer, rectal cancer, breast cancer, and head and neck tumors. In colon cancer, the high expression of OX40 is positively correlated with improved overall survival, but this positive correlation depends on the staging of the tumor. In lymphoma, the expression of OX40 on the surface of tumor-infiltrating Treg cells was dynamically increased. It is worth noting that the level of OX40 mRNA in breast cancer is related to anti apoptosis, immunosuppression and promoting the expression of tumor mRNA gene markers. OX40L on platelets may play an antagonistic role in cancer and anti-tumor immunity.

5.1. Basic Research of OX40/OX40L

The activation of the OX40/OX40L pathway can enhance the activity of CD4⁺T cells and CD8⁺T cells. In addition, it can also inhibit the function of Treg (such as weakening the inhibitory effect of Treg cells or depleting Treg cells). Research has found that the OX40/OX40L signaling pathway can be induced and activated in mice through various methods, such as specific OX40 antibody agonists, OX40L-Fc fusion protein, dendritic cells (DC), etc.[7, 8]. OX40 antibodies or OX40 fusion proteins exhibit strong anti-tumor effects on tumors with lower load in mice. However, for larger or metastatic tumors, the therapeutic effect of OX40 antibody monotherapy has certain limitations. Therefore, adopting different combination therapy strategies may overcome resistance to OX40 antibody monotherapy. Due to the significant prolongation of the survival time of antigen-activated CD4⁺ and CD8⁺T cells through the OX40/OX40L signaling pathway, it is possible to combine OX40 agonists with some therapies that increase antigen loading. Therefore, OX40 agonists can be considered in combination with targeted inhibitors, cancer vaccines, immune checkpoint antibodies, etc. OX40L-Fc combined with chemotherapy and vaccine for the treatment of glioma. OX40 antibody can prolong the survival time of T cells transferred to the body, providing a basis for its combination with adoptive T cell therapy. The combination of OX40 agonists and immunosuppressants can exert a synergistic effect in the treatment of metastatic malignant tumors. The research results of ovarian cancer models show that the combination of PD-1 inhibitors and OX40 agonists can significantly prolong animal survival time, with 50% of experimental animals experiencing tumor regression on the 90th day. Similarly, intratumoral injection of TLR9 agonists, CTLA-4 inhibitors, and OX40 agonists can inhibit tumor growth, even effective against brain tumors.

5.2. Clinical Studies of OX40/OX40L

OX40/OX40L, as a new tumor immunotherapy target, is still in the early stages of clinical research. As of now, dozens of drugs targeting OX40 are in the clinical research stage, mainly used for the treatment of metastatic tumors (Table 1) [9-10]. The mouse monoclonal anti-human OX40 agonist antibody 9B12 (MEDI0562) is the first OX40 antibody used in human studies. This experiment evaluated the toxicity of 9B12 by administering three doses of 9B12 intravenously to 30 patients with metastatic tumors. The results showed that patients had good tolerance, and after three doses, at least 30% of patients showed tumor shrinkage with at least one metastatic lesion. And significant immune regulatory activity was observed, including an increase in T cell activation and proliferation. A clinical trial of combined therapy for metastatic prostate cancer (NCT01303705), which induced the proliferation of memory T cells and effector T cells using OX40 antibodies, as well as cyclophosphamide and radiation induced tumor antigen release to promote immune response to cancer. In addition, clinical studies on the combination of OX40 antibody and CTLA-4/PD-L1 antibody, OX40 antibody and CD20 antibody, as well as the combination of OX40 antibody with

radiation therapy and surgical treatment, are still ongoing. We look forward to the disclosure of more trial results.

Table 1: Clinical research and development status of anti-tumor drugs targeting OX40/OX40L.

| Drug name | Clinical stage | Tumor category | Clinical trial number |
|-------------------------------|----------------|---|--|
| HS-130 | I | Advanced solid tumor | NCT04116710 |
| MEDI6469 | Ib | Advanced cancer | NCT02274155/ NCT01303705/ NCT02205333 |
| 9B12 | I | Advanced cancer | NCT01644968 |
| PF-04518600 | Ib or II | Recurrent/refractory acute myeloid leukemia | NCT03390296 |
| SL-279252 | I | Advanced solid tumors | NCT03894618 |
| PF-04518600 | I | Advanced or metastatic cancers | NCT02315066 |
| MOXR0916 | I | Advanced solid tumors | NCT02219724/ NCT02410512 |
| MEDI6383 | I | Recurrent or metastatic solid tumors | NCT02221960 |
| MEDI0562 | | Advanced solid tumors | NCT02318394 |
| ATOR-1015 (Bispecific mAb) | I | Advanced cancer | NCT03782467 |
| BMS-986178 | I or IIa | Advanced cancer | NCT02737475 |
| GSK3174998 | I | Advanced solid tumors | NCT02528357 |
| MEDI0562 | I | Advanced solid tumors | NCT02705482 |
| INCAGN01949 | I or II | Advanced cancer | NCT02923349/ NCT03241173 |
| PF-04518600 | II | Metastatic renal cell carcinoma | NCT03092856 |
| mRNA-2416 | I/II | Relapsed/refractory cancer | NCT03323398 |
| DNX-2440 | I | Resectable liver metastasis | NCT04714983 |

6. Summary and Outlook

CTLA-4, PD-1, and PD-L1 as first-generation immune checkpoint inhibitors have shown significant advantages over traditional treatments in tumor treatment. However, the emergence of drug resistance and limited objective response rates remind us that the road to overcoming tumors is still long, and more immune targets need to be explored. OX40/OX40L is a novel pair of co-stimulatory molecules in the immune response of the body, which plays a significant role in tumorigenesis and progression. It can activate effector cells, deplete Treg cells, and activate the immune system through various mechanisms. Preclinical animal studies have shown that OX40 antibodies have broad prospects in the treatment of malignant tumors alone, as well as in combination with other treatment methods such as immunomodulators, vaccines, surgery and radiation therapy. However, more basic research is needed to comprehensively demonstrate the biological characteristics of the OX40/OX40L in order to provide new strategies for immunotherapy of malignant tumors.

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