OX40 (CD134) and OX40 ligand, important immune checkpoints in cancer

Abstract: Immunotherapy has shown promising results in cancer treatment. Research shows that most patients might be resistant to these therapies. So, new immune therapies are needed. OX40 (CD134) and OX40 ligand (OX40L), costimulatory molecules, express on different types of immune cells. The interaction between OX40 and OX40L (OX40/OX40L) induces the expansion and proliferation of T cells and decreases the immunosuppression of regulatory T (Treg) cells to enhance the immune response to the specific antigen. For the important role OX40 takes in the process of immunity, many clinical trials are focusing on OX40 to find out whether it may have active effects in clinical cancer treatment. The results of clinical trials are still not enough. So, we reviewed the OX40 and its ligand (OX40L) function in cancer, clinical trials with OX40/OX40L and the correlation between OX40/OX40L and other immune checkpoints to add more ideas to tumor feasible treatment.

Keywords: cancer, immune checkpoints, OX40/OX40L, immunotherapy

Immunotherapy has shown promising results in cancer treatment, cancer immune checkpoint blockades also have got good results. It was demonstrated that combining cancer vaccines or checkpoint inhibitors with different immunotherapeutic agents could augment the anti-tumor effects and get better results in cancer patients.

Tumor necrosis factor receptor superfamily member 4 (OX40) (CD134) and OX40 ligand (OX40L) (CD134L) (CD252) are on chromosome 1. The OX40 and OX40L could be expressed by endothelial cells, mast cells, activated natural killer (NK) cells, dendritic cells (DCs), B cells, microglial cells, activated T cells and Foxp3+ regulatory T cells. OX40L could initiate OX40 signals in activated T cells. OX40L on T cells could provide signals via the interactions between T cells and upregulate the anti-apoptotic protein on T cells to enhance T cell survival, cytokine production and induce the CD4 memory T cell expansion. The co-stimulation in B cells through the OX40/OX40L pathway contributed to CD4 cell generation, survival and T helper 2 (Th2) development. OX40/OX40L could promote NK cell activation, cytokine production and cytotoxicity and enhance targeted cells lysis. Mast cell via the OX40/OX40L pathway could induce T cell proliferation. OX40 on Treg cells played an important role in Treg cell development and homeostasis. We made a figure to clarify the function of OX40-OX40L pathway (Figure 1).

OX40/OX40L and diseases

Many diseases were associated with OX40/OX40L, so many researchers focused on it to find new way of treatment. The activation of OX40 promoted the generation...
and expansion of activated T cells and memory T cells, thus aggravating autoimmune diseases like Graves’ disease, autoimmune arthritis and uveitis.\textsuperscript{24–27} OX40 was critically important in sustaining the anti-viral immune response during the viral infection.\textsuperscript{19,28–30} OX40–OX40L signaling increased the adaptive immune response to an allograft by promoting effector and memory T cell survival. And blockade of OX40–OX40L interaction could decrease the T cells infiltration in the targeted organs to prevent allograft rejection.\textsuperscript{31–34} OX40L could promote the inflammatory cells infiltration into lesional tissues, leading to the pathological fibrosis in skin and internal organs. And blocking OX40–OX40L regressed the fibrosis.\textsuperscript{35,36} OX40–OX40L interaction on immune cells might contribute to idiopathic inflammatory myopathies through different pathways in the inflamed muscle.\textsuperscript{37} OX40/OX40L pathway was involved in the pathological process of Crohn’s disease (CD). And blockade anti-OX40 might be beneficial for the treatment by controlling the T cell-mediated inflammatory in vivo.\textsuperscript{38,39} Data implicated that OX40/OX40L participated in pathophysiology of acute myeloid leukemia and also enhanced NK cell cytotoxicity.\textsuperscript{18}

**OX40/OX40L and cancer**

OX40 was expressed on the tumor-infiltrating lymphocytes (TIL) in head and neck squamous cell carcinoma, ovarian cancer, gastric cancer, cutaneous squamous cell carcinoma, breast cancer and colorectal cancer.\textsuperscript{40–45} Agonistic anti-OX40 antibodies had anti-tumor effects.\textsuperscript{46–52} OX40 triggering regressed Treg cells, allowing DCs to reach the draining lymph nodes and prime the specific CD8 lymphocytes response to the tumor.\textsuperscript{48,53} Many research focused on the anti-tumor immunotherapy, based on activating costimulatory molecules OX40 and OX40L. Here, we showed some of them (Table 1).

**Clinical trials of OX40/OX40L**

Based on the role of OX40 and OX40L in the immune system, more and more research focused on its therapeutical effects. Many companies detected the immune checkpoints OX40 and OX40L, searching for the new approaches to treat tumors and autoimmune diseases, many of which are now making great advance in clinical development (Table 2). The results of clinical trials showed the OX40, as a potent immune-stimulating target, played an important role in anti-tumor therapy. The agonist anti-OX40 increased CD4 FoxP3\textsuperscript{−} and CD8 T cells proliferation and the response to the tumor-specific antigen, enhancing both humoral and cellular immunity in cancer treatment.\textsuperscript{49}

**Correlation of OX40/OX40L and other immune checkpoints**

The results of studies suggested that some diseases were not sensitive to antibody therapy alone. So, it was necessary to study on the relationship between checkpoints to work out more effective treatment. CTLA-4, a molecule on T cells, inhibited the proliferation of T cells and cytokine production, thus limiting the lymphocyte immune reaction.\textsuperscript{68–72} Anti-CTLA-4 blockade induced the depletion of Treg cells within tumor and activation of Teff cells.\textsuperscript{71,73–76} Combining agonist anti-OX40 and antagonist anti-CTLA-4 further enhanced CD4 and CD8

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**Figure 1** OX40–OX40L interaction model. **Abbreviations:** Th2, T helper 2; NK, natural killer; TCR, T cell receptor; MHC, major histocompatibility complex; APC, antigen presenting cell.
T cells responses to antigen, indicating they had synergistic effects in improving tumor regression.\(^77\)–\(^79\) And the cytokine of Th1 and Th2 CD4 T cells increased significantly.\(^64\) Whether the combination therapy altered the suppressive function of Treg cells remained deeper exploration.\(^63\),\(^64\) The combination was still more than the sum of its part.\(^80\)

Programmed death-1 (PD-1) is a molecule that suppresses the immune reaction, inducing T cell exhaustion and apoptosis. Programmed death-ligand 1 (PD-L1), expressed on tumor cells or other tumor-related immune cells, could suppress anti-tumor immune response.\(^81\)–\(^84\) The function of PD-1 and PD-L1 was affected by the complex immunoregulation. PD-1 blockade had already been used in cancer treatment and got a satisfying result.\(^82\),\(^84\) It was reported that PD-1 inhibitor added at the initiation of the cancer treatment could reduce the effects ofOX40 agonist antibody, for it might cause the antigen-specific CD8+ T cell diminishment.\(^85\) And timing of PD-1 blockade using might determine whether it was effective immunotherapy when combined withOX40 therapy.\(^81\) In most cases,OX40 agonist and PD-1 blockade had a synergistic effect in disease treatment. OX40, combined with CD27 mediated co-stimulation, could synergize with PD-L1 inhibitor by activating CD8+ T cells.\(^86\) Combining OX40 stimulation and PD-L1 blockade could synergistically augment hepatitis B virus (HBV)-specific CD4 T cell responses by promoting Th cells to secrete IFN-\(\gamma\) and IL-21 in patients with HBV infection.\(^87\) In some poorly immunogenic tumors, combining PD-1 blockade and OX40 stimulation had an anti-tumor effect by inducing cytotoxic T lymphocyte, increasing the Teff cells and decreasing the immunosuppressive cells, while individual did not.\(^41\)

\(4-1BB\) (CD137), member of the TNFR family enhanced T cell proliferation, effector function and cytokines production, and induced maturation of DC, thus increasing the immune reaction.\(^88\)–\(^93\) Agonistic anti-\(4-1BB\) immunotherapy was active against intracranial glioma.\(^66\) The costimulatory pathway of OX40–OX40L and \(4-1BB\)-\(4-1BB\) L functioned independently to enhance immune cells response.\(^88\) The combination of OX40 agonist and \(4-1BB\) agonist induced profound expansion of CD8 T cell.\(^96\),\(^97\) But the response of CD4 T cell to the dual costimulation seemed to be additive instead of synergistic.\(^98\) On the whole, the combination therapy

| Disease | Finding | References |
|---------|---------|------------|
| Cancer | Anti-OX40L delayed the tumor progression and even eradicated tumors. | 54 |
| Breast cancer | Activation of OX40 receptor+ CD4+ T cells could stimulate the anti-tumor immune response in mammary cancer. | 55 |
| Colon cancer | High levels of OX40 positive lymphocytes were correlated with better survival in colon cancers. | 56 |
| Cancer | OX40L fusion protein could inhibit the tumor by direct intra-tumor injection. | 9 |
| Cancer | OX40L-transduced tumor cells could elicit tumor-specific Th1 immune responses, generate anti-tumor immunity and inhibit the tumor growth in vivo. | 57 |
| Cancer | OX40 agnostic therapy contributed to anti-tumor CD8 effector T (Teff) cells priming and enhanced CD8 T cell response to the antigen tumor derived. | 58–60 |
| Cancer | Intra peritoneal injection of OX40L-immunoglobulin fusion protein could inhibit tumor growth. | 61 |
| Cancer | OX40L on DCs could induce anti-tumor immunity via binding OX40 on CD4+ T cells and NK T cells. | 62 |
| Advanced cancer | Agonistic anti-OX40 increased circulating T cells, B cells and intratumoral Tregs, enhancing tumor-specific immune responses. | 49 |
| Cancer | Agonist anti-OX40 therapy combined with cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) blockade augmented antigen-specific CD8 T cells and limited the Th2 cells polarization, eliciting potent anti-tumor immunity. | 63,64 |
| Cancer | OX40 agonistic and IDO (indoleamine-(2,3)-dioxygenase) inhibitor produced a synergistic effect on the tumor immune response. | 65 |
| Glioma | Agonist anti-OX40 immunotherapy was active against intracranial glioma. | 66 |
| Metastatic ovarian cancer | Combining anti-OX40 and anti-CD73 immunostimulants increased cytotoxic T cell infiltration and decreased tumor promoting immune cells. | 67 |

**Abbreviations:** NK, natural killer; DCs, dendritic cells; Th2, T helper 2.
could synergistically inhibit cancer by producing more enhanced signals. 98,99

**Summary**

Immune checkpoints play vital roles in cancer treatment. It was proved that the agonist anti-OX40/OX40L could enhance anti-tumor response by promoting the function of immune cells. More and more researchers focused on OX40/OX40L in cancer immunotherapy. But until now, the effects of OX40/OX40L treatment are still limited. Researchers are devoted to combine OX40/OX40L with other immune checkpoints in cancer treatment, which had also made some achievements, but the mechanisms of the synergy between OX40/OX40L and other immune checkpoints still need to be further studied.

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**Disclosure**

The authors report no conflicts of interest in this work.

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