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Review

Role of OX40 and its ligand as costimulatory modulators in cancer immunotherapy

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Abstract: Body's defence mechanism has ability to combat tumour cells but tumour cells can circumvent immune system in order to flourish. Therefore, current research focuses on reinvigorating immune system to combat against extensive range of human malignancies through immunotherapy. Recently, immuno-therapy has demonstrated beneficial outcomes in cancers treatment but the main drawbacks are primary and acquired resistance to the therapeutic agents and immune-related toxicities. Therefore, novel immune therapies are direly required. Co-stimulatory molecules such as TNF Receptor Superfamily Member 4 (OX40, CD134) and its ligand TNF Superfamily Member 4 (CD252, OX40L) are expressed on different immune cells. The mutual interaction between OX40 and its ligand (OX40/OX40L) decreases the functional capacity of immunosuppression offered by regulatory T cells (Tregs) and induces the proliferation of T cells against specific antigen enhancing the immune response. Many clinical trials are focusing on OX40/OX40L therapeutic agents to find out whether they have therapeutic effect on cancer treatment. The initial phase trials result of OX40 and its ligands focusing therapeutic agents are encouraging but still not sufficient. This review will concentrate on the cellular and molecular pathways of OX40-mediated T-cell co-stimulation, the expression of OX40 and OX40L in tumours, the implications of their interactions and their under-or over-expression patterns, with particular focus on the function of OX40 in tumours of different origins. Finally, we discuss results of clinical trials of OX40 and OX40L directed pharmacotherapy and the lacunae that need to be filled.

Keywords: OX40; OX40 ligand; T-cells; immunotherapy

Abbreviations: APCs: antigen presenting cells; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; FOXp3: forkhead box P3; HCC: Hepatocellular carcinoma; MHC: major histocompatibility complex; NK: Natural Killer cell; NSLC: Non-small cell lung carcinoma; OSCC: Oral squamous cell carcinoma; OX40: Tumour necrosis factor receptor superfamily member 4; OX40L: Tumour necrosis factor superfamily member 4 ligand; TCR: T cell receptor; Tregs: Regulatory T cells

1. Introduction

Cancer biology and cancer therapeutics have experienced paradigm shift since the concept of "hallmarks of cancer" has evolved. The notion involves certain characteristics acquired by tumour cells based on their heuristic approach altering the common molecular mechanisms aiding them to transform, proliferate and metastasize [1]. Additionally, in the past few decades, the crucial pathways involved in oncogenesis are unraveled, giving better insight into cancer biology for precise targeting of carcinogenesis. The rationale behind being characteristics that are common in different types of cancers and within tumour cells [2].

Among the hallmarks of tumourigenesis "tumour evasion" is of prime significance. The tumour cells possess atypical genetic and epigenetic alterations distinct from normal cells of the host. As a normal phenomenon, these alterations present as antigens; stimulating the natural killer cells and T cells to inhibit the growth of cancer cells. However, the clever tumour cells can mold the immune system escaping the defense mechanism favoring proliferation and progression of tumour [1]. Tumour evasion involves certain mechanisms such as malignant cells presenting as weak stimulators of immune response, additionally impairing the adaptive immune response by altering the signaling and functionality of antigen presenting cells (APCs) as well as T cells. Researchers have put their efforts in order to gear up the immunity against these clever cancer cells in the shape of immunotherapy [3]. Recently, immunotherapy and cancer immune checkpoint blockades have shown promising results in treating different cancers. The immune checkpoint blockers (ICB) have shown their favorable outcomes in earlier phase and adjunctive care, especially in melanoma and non-small cell lung cancer. These agents focusing on ICB are now part of routine treatment in metastatic tumours [2]. Although, a proportion of ICB recipients due to primary resistance do not reap benefits of this therapy as well as onset of acquired resistance results in tumour progression [4]. However, combinations of various immunotherapeutic agents have been shown to increase anti-tumour effects and yield improved outcomes in treating cancer patients. Despite the results, newer immune therapies are urgently needed. The costimulatory molecules such as "*tumour necrosis factor receptor superfamily member 4*" (OX40; CD134) and its binding ligand (OX40L) are under the research for augmenting the immune system. They act by interacting with each other leading to the suppression of regulatory T cells (Tregs) thus declining the immunosuppression imposed by them. On the other hand, augmented immune defense against a specific antigen via proliferation of T cells and memory cells [5]. This review will concentrate on the cellular and molecular pathways of OX40-mediated T-cell co-stimulation, the expression of OX40 and OX40L in tumours, the implications of their interactions and their under or over-expression patterns, with particular focus on the function of OX40 in malignancies of different origins. Finally, we discuss results of clinical trials of OX40-OX40L directed pharmacotherapy and the lacunae that required to be filled.

2. Methodology

Review of scientific literature was performed in the Scopus, PubMed, Web of Science and Embase databases for studies published between 1990 and 2021. The search strategy was executed via keywords: OX40; OX40 ligand; T-cells; Immunotherapy. The advanced search was performed using Boolean operators ('AND', 'OR', 'NOT') with the combination of words or phrases and titles; in addition, reference lists from all relevant reviews and key publications were scanned manually for appropriateness, relevance to the topic and additional references.

3. Costimulatory molecules

The T cell activation mechanism entails interaction between the T cell receptor (TCR) and the antigenic MHC/peptide complex. The resulting response is not cogent and sustainable, thus requiring signaling by co-stimulatory molecules for optimal priming, production, and differentiation of T-cells to generate robust immune response [6].

The costimulatory molecules are primarily classified into two groups as illustrated in Figure 1.

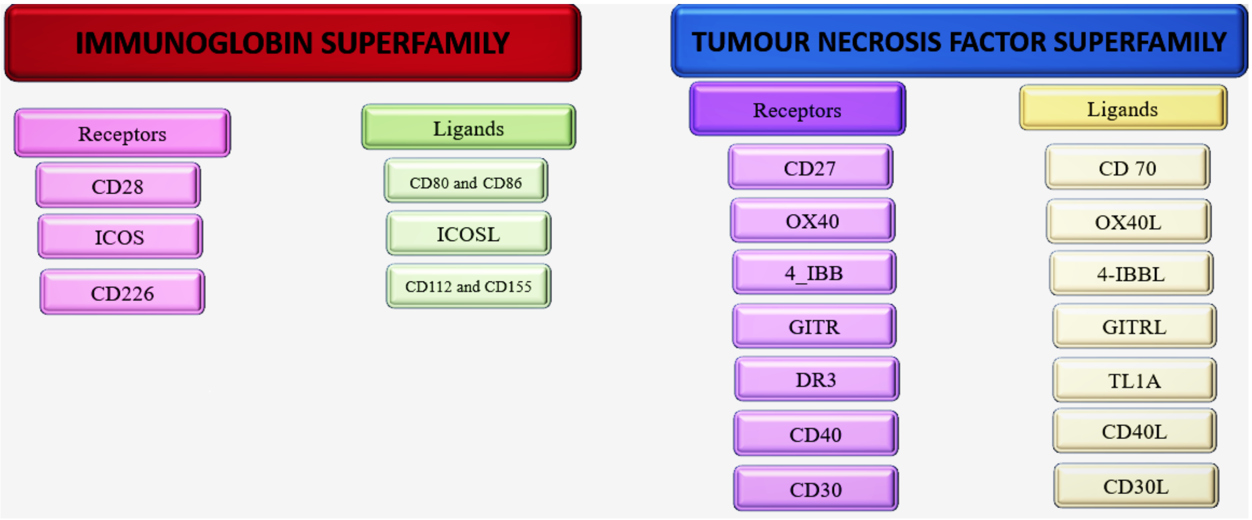

Figure 1. Classification of co-stimulatory molecules. ICOS: Inducible costimulatory; OX40: Tumour necrosis factor receptor superfamily member 4; OX40L: Tumour necrosis factor ligand superfamily member 4; 4-IBB: Tumour necrosis factor receptor superfamily member 9; GITR: Glucocorticoid induced TNFR related protein; DR3: Death receptor 3; TL1A: Tumor necrosis factor-like cytokine 1A.

4. OX40 and OX40L

The costimulatory molecules "tumour necrosis factor receptor superfamily member 4" (OX40) (CD134) and "OX40 ligand" (OX40L/CD134L/CD252) genes are found on chromosome one [7]. The newly formed T lymphocytes do not have expression of OX40 and its ligand; their expression initiates to occurs when major histocompatibility (MHC) peptide connects with T cell receptor (TCR) leading to stimulation of T cells. Subsequent activation by antigen along with ligation of CD86 with CD28 and CD40 with CD40L are crucial steps in activation of T cells and thus, expression of OX40 and its ligand [5,8]. OX40 is expressed on activated T cells (CD4 and CD8) primarily and lower expressions are reported on natural killer cells (NK) and natural killer T cells. The ligand of OX40, OX40L (CD134L or CD252), which is also known as "tumor necrosis factor superfamily member 4 (TNFSF4)", is expressed on antigen-presenting cells (APCs), i.e., dendritic cells (DCs), B cells, and macrophages predominantly, and is also expressed on activated T cells (CD4 and CD8), vascular endothelial cells and mast cells [8].

4.1. OX40 and OX40L signaling pathway

The interaction of OX40 with OX40L enhances the immune system by functioning on two levels. One of the functions is reduced transcription of Forkhead box P3 (FOXP3) and cytotoxic T lymphocyte associated protein 4 (CTLA-4). Thus, ultimately the decreased transcription of FOXP3 and CTLA-4; the main modulators of T regulatory cells (Treg) and the negative immune regulator respectively, leads to decreased function of Treg cells leading to decreased immunosuppression provided by the immune system [9]. The other aspect is the increased transcription of antiapoptotic gene (Bcl-2, Bcl-X) in T cells (CD 4 and CD 8) as well as of survivin, which functions to decrease levels of caspases involved in the apoptotic pathway as shown in Figure 2. These functions lead to accentuated T cell survival, increased cytokine production together with enhanced cytolytic activity consequently, increased antitumour activity as well as memory T cell generation [10,11]. Similarly, the pathway of these costimulatory immune modulators contributes to NK cell activation, enhanced cytotoxicity, increased cytokine production and targeted cell lysis [12–14].

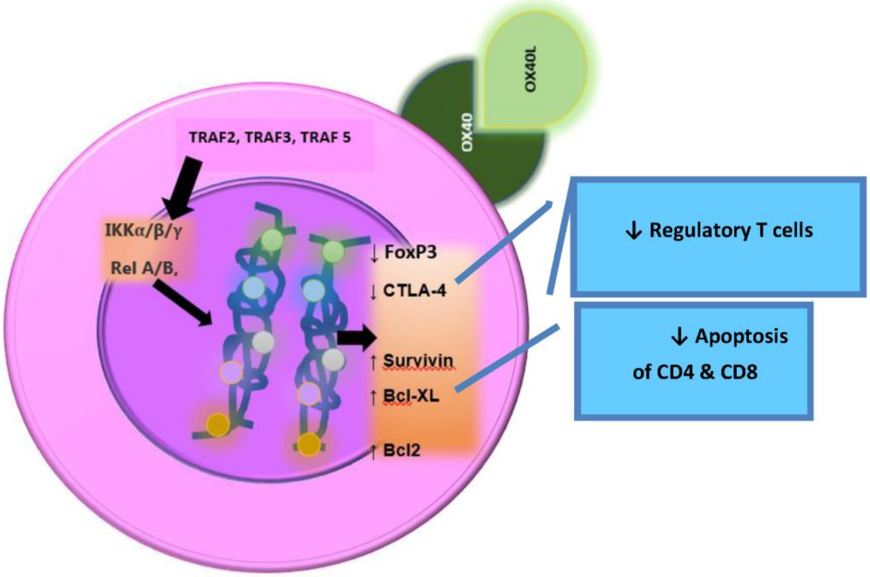

Figure 2. Proposed signalling pathway of OX40 and its ligand. OX40 and OX40L interaction recruits TRAF2, 3 and 5 which activate the signals through IKKα/β/γ and Rel A/B in the nucleus, thus causing anti-apoptotic genes up-regulation such as: Bcl-2, Bcl-XL and survivin along with down-regulating: Foxp3 and CTLA4.

4.2. OX40 and OX40L expression in different tumours

Co-stimulatory mediators' involvement is reported to be associated with many non-cancerous conditions such as autoimmune disorders and allograft rejection as well as in malignancies. Literature supports the costimulatory molecules enacting pathophysiology and progression of tumours such as hematological malignancies (acute myeloid leukemia) as well as other solid tumours. Expression of OX40 was observed in the tumour-infiltrating lymphocytes (TILs) of oral and cutaneous squamous cell carcinoma along with cancers of ovary, breast, lung, gastric and colorectal regions [15–20]. OX40L expression has also been investigated in head and neck cancer, lung adenocarcinoma and hepatocellular carcinoma [17,21–23]. The logic behind evaluating these markers in tissue biopsies is that it may truly reflect the expression levels in the tumour milieu, but it is ethically and clinically improper to repeat biopsies to evaluate alterations in their expression with disease progression or regression or during or after the treatment.

On the other hand, the expression patterns vary among different malignancies in context to the severity of the disease, for instance, higher expression of OX40 was observed in ovarian cancer (n = 47) along with better prognosis and chemosensitivity [15]. Likewise, higher expression of OX40 (n = 100) in TILs of non-small cell lung carcinoma (NSCLC) demonstrated favorable prognosis in addition to augmented levels of Interferon-gamma (IFN-γ) in surgically resected patients of stages I-III [17]. Interestingly, another study of NSCLC showed lower expression of OX40 in TILs (n = 139) and it was related to longer recurrence free survival (RFS), overall survival (OS) and better prognosis [21]. The previous study contradicts with the research of Massarelli et al., presented. Higher expression of OX40 or OX40L in tumours such as breast carcinoma (n = 40, qPCR in blood samples), colorectal carcinoma (n = 22) and hepatocellular carcinoma (n = 370, RNA–sequencing; n = 316, IHC) were also observed in advanced stages and associated with reduced survival rate [16,20,23]. A research in gastric carcinoma revealed lower expression of OX40 on tissue biopsies in advanced stages [19]. Similarly, lower expression of OX40L mRNA was associated with worse prognosis in patients suffering from melanoma [22].

The studies on tumours of head and neck region have also reported variable results. A study on oral squamous cell carcinoma (OSCC) patients (n = 18) conducted by Pramita Baruah et al. reported significantly lower levels of OX40 on CD4+ T cells in advanced stages of tumours, i.e., T3 and T4 compared to early carcinomas, i.e., T1 and T2 [24]. Montler et al. reported (n = 29) higher frequency of OX40 expression in the Treg TIL population compared with peripheral Tregs [25]. While Lecerf et al. (n = 96) reported higher OX40L mRNA in 74% of patients and associated with unfortunate outcomes after the surgery [18]. These researches signify the fact that every cancer is different and the risk factors associated with cancer also differ among individuals. Therefore, the notion of targeting every cancer with few common treatment modalities seems contentious. Moreover, data on gene expression in blood and serum levels is lacking, which needs to be explored that may give useful insight of differences of their expression in blood and the tumor level for better understanding and prescription of OX40 and OX40L based future therapeutic agents in different cancers. We are of the opinion that blood and serum samples are easier to obtain and can be taken repetitively, reflecting the whole-body immune status of the cancer patient and to evaluate the future drugs effect.

4.3. Drugs targeting OX40 and OX40L in cancers

Based on the biological rationale and functions of OX40 and its ligand, agonists of these markers are being explored in treatment of different cancers (Table 2). Effective therapeutic responses with OX40 agonists are observed in various preclinical cancer models created in mice, including melanoma, glioblastoma multiforme (GBM), breast cancer, colon carcinoma, renal and prostatic cancer and lung carcinoma also seen in chemically induced sarcomas [29,30]. The clinical trial (NCT02274155) reported preoperative MEDI6469 (OX40 agonist) administration was safe and resulted in augmented stimulation and increased production of T cells within the tumour in 2 weeks following infusion of OX40 agonists [31]. Another trial reported enhancement of humoral and cellular immunity with an OX40 agonist Ab, hence, exhibiting antitumour activity [32]. Trial of PF-8600 (humanized agonist IgG2 monoclonal antibody to OX40) in HCC demonstrated safety and efficacy along with durability of stable disease (17–18 months) in half of the study subjects [33]. However, the results in animal studies due to methodologically inadequate designed studies have not been replicated well in humans and that is a major hindrance in the success of drugs development. Griffith et al. recently investigated a panel of anti-human OX40 antibodies (anti-hOX40 mAb) and evaluated their *in vitro* activity and binding activity. Anti-hOX40 mAb were then tested in a variety of *in vivo* models, concluding that targeting a specific isotype and domain binding region can influence the mechanism of action and effectiveness of such antibodies [34].

Table 2 depicts the effect of monotherapy and different combined therapies on different cancers. The anti-OX40 agonist increased expansion of CD4, FoxP3^{-} and CD8 T cells [35]. Other ICB therapeutic agent's combination augmented anti-tumour response synergistically by promoting the function of immune cells. However, the costimulatory molecules are only produced for a short period of time after stimulation. Another barrier to this type of treatment is that repetitive agonistic T cell activation might lead to immune exhaustion [36].

Thus, immune checkpoints based therapeutic agents play a vital role in cancer treatment and, on the whole, combinations of agents targeting OX40 along with other modalities of remedy are under evaluation. However, this combination should be targeted by keeping in mind the immunogenicity of the tumour as well as on the mediators involved in diverse pathways of immune stimulation along with the schedule and time lines of particular drug administration in combinatorial strategies. Additionally, the appropriate drug design targeting appropriate isotypes to elicit potent T-cell agonism and antitumour activity. Though, studies are still limited to reach some conclusion about whether monotherapy or combination therapies are able to alter the functions of Treg and other T cells in different cancers. Furthermore, in order to minimize the translational failure, in-depth evaluation of the effects of these agents *in vivo* is required as well as recognition of logical combination approaches that can be tested in the clinics with success.

Table 1. Expression of OX40/OX40L in different tumours.

Tumour	n	Technique	Conclusion
OSCC	18, 12 (controls)	Flow cytometry, cell lines	OX40 levels were decreased on CD4+ T cells. Lower levels of OX40 in late-stage tumours (III/IV) compared to early-stage (I/II)[24].
OSCC	29	Flow cytometry (blood and tissue)	OX40 was over-expressed in Treg TIL's population compared with peripheral Tregs [25].
OSCC	96	qPCR and IHC	OX40L mRNA overexpression in majority (74%) of cases and poor outcomes after the surgery [18].
OSCC	78	ELISA	Higher serum levels were observed in advanced stages [26].
Breast Ca	40	qPCR (blood samples)	Increased expression of OX40 in later stages [21].
Ovarian cancer	47	IHC	Higher OX40 expression was associated with better recurrence free survival (RFS) and chemosensitivity [15].
Hepatocellular carcinoma (HCC)	316, 370	IHC, RNA-seq	Higher expression of OX40 was related with augmented serum levels of AFP, vascular invasion, poor prognosis [23].
HCC	34	qPCR	Higher expression of OX40 mRNA significantly correlated with the degree of tumor differentiation while lower OX40Lexpression was observed in tumor tissue [27].
Gastric cancer	24	IHC	Reduced levels of OX40 in advanced stages, i.e., III and IV [19]
Colorectal cancer (CRC)	22	qRTPCR, ELISA	Higher OX40 blood levels were correlated with a poor prognosis [20].
Non-small cell lung cancer (NSCLC)	139	IHC	Lower expression of OX40 was related to better prognosis, longer RFS and OS [21].
Non-Small cell Lung Cancer	100	IHC	Favorable prognosis and increased levels of IFN-gamma were observed in surgically treated patients with higher expression of OX40 in the TIL's [17].
Advanced Lung adenocarcinoma	56	IHC and ELISA	Higher serum OX40 and OX40L levels had poorer prognosis [28].

Table 2. OX40/OX40L therapeutic agents and effects on different cancers.

Therapeutic agents	Type of tumour	Research model	Effect
"OX-40R-specific mAb (termed *OX-86*)"	Fibrosarcoma, Melanoma, Glioma and pulmonary metastasis of fibrosarcoma	Mice	Anti OX40 delayed the tumour progression and even eliminated tumours such as fibrosarcoma. However, pulmonary metastasis of fibrosarcoma and melanoma did not respond to the therapeutic agent [37].
OX40L soluble form	Breast carcinoma	Mice and human	This led to upregulation of OX40R on CD4+ T cells in TILs and Tumour draining lymph node cells [38].
"Agonist anti-OX40 antibody combined with anti-PD-1"	ovarian cancer	Mice	PD-1 blockade and OX40 activation act synergistically and protect against tumour growth [39].
"Agonist anti-OX40 antibody combined with anti-PD-1"	model of mammary cancer (MMTV-PyMT)	Mice	This combination therapy induced more tumour regression in comparison with monotherapy and enhanced tumour-specific T cells [40].
"Anti-OX40 plus anti-CTLA-4 plus HER2 vaccine"	Mammary cancer model	Mice	The combination reverted T cell anergy, increased lifespan of memory T cell response and enhanced CD8+ T cell effector function [41].
"Bispecific antibody targeting CTLA-4 and OX40 (ATOR-1015)"	Normal cell lines of Ovary and Kidney Cell lines of Bladder, pancreas, and colon Cancer	Cell lines of human and mice	Induced stimulation of tumour-specific T-cell and depletion of regulatory T cells plus amplified long-term immunological memory [42].
"Recombinant adenovirus vector expressing mouse OX40L (AdOX40L)"	Melanoma, Colorectal, Lung adenocarcinoma	Mice	It showed significant suppression of tumour growth in tumour-bearing mice along with survival advantages when injected directly to the tumour site [43].

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Therapeutic agents	Type of tumour	Research model	Effect
"mOX40L fusion protein"	experimental lung metastasis and colon (CT26) and breast (4T1) carcinomas	Mice	Intraperitoneal injection inhibited experimental lung metastasis. This combination was effective in inhibiting colon and breast cancer [44].
"GVAX and systemic agonist anti-OX40 monoclonal antibody	Glioma	Mice	The combination improved survival [30].
"GVAX, anti-PD-1 monoclonal antibody, and agonist anti-OX40 monoclonal antibody"	Glioma	Mice	Enhanced CD4 and CD8 amongst the TILs with decreased proportion of Tregs. consequently, emphasizing increased antitumor activity [45].
Indoximod and anti-OX40 agonist	Lung metastasis	Mice	Combination led to an increment of effector T cells infiltrating the tumour and their increased specificity and functionality [46].
Agonistic murine antibody to OX40 (MEDI6469)	Head and neck squamous cell carcinoma (n = 17)	Human	Pre-operative administration was safe and triggered the increased activation and proliferation of T cells within the tumour [32].

5. Conclusions

Currently, the focus of cancer treatment is targeting the biological hallmarks of cancers. The functions of OX40 and OX40L are well documented in enhancing the immune system. OX40 and its ligand targeted therapy, on the other hand, have shown beneficial outcomes in various tumor animal models, but translational failure has been observed in clinical trials. The preliminary clinical reports suggest that immunotherapy efficacy in humans is limited. However, OX40 co-stimulation is a potential approach for use in conjunction with immunotherapies that target other immune check points or other treatment modalities. Although, paramount importance is that the biological rationale and basic level data is lacking, which is also hampering drug development and its success. In future, knowledge of expression levels in tumour tissue and in blood in regards to grading and staging would help in understanding the rationale for devising such therapeutic agents and their combinations. Additionally, we suggest the tumour mutational burden, the immunogenicity of different malignancies, drug design and the scheduling of combinatory treatments should also be assessed. The expression levels, mutational levels and associated biomarkers can be strong predictors of the severity and activity of different cancers and their response to the therapies.

Conflict of interest

All authors declare no conflict of interest in this paper.

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