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Review

Interaction of opioid growth factor (OGF) and opioid antagonist and their significance in cancer therapy

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ABSTRACT

Endogenous opioids are neuro-peptides with multifunctional properties. Historically, opioids are used to mediate pain; however, excess opiate consumption can lead to addiction. One endogenous opioid, methionine enkephalin (MENK), was reported to modulate cell growth, MENK was identified as an opioid growth factor (OGF) that interacts with the OGF receptor (OGFr) and regulates cell proliferation. Further, opioid antagonists, including naltrexone and naloxone are widely used to reverse drug and alcohol overdoses. Naltrexone (NTX) acts on all opioid receptors, blocking the interaction between OGF and OGFr, and thus influencing cell growth. During the last decades, insights have been made concerning the interaction between OGF and OGFr, confirming that both opioids and opioid antagonists have an important role in balancing host homeostasis, host immunity and mediating cancer therapy. This review provides insight into the interactions between OGF and OGFr in the treatment of cancers.

1. Introduction

1.1. Opioid growth factor (OGF)

Widely distributed, endogenous opioids with neurotransmitter functions are derived from 3 prohormones: proenkephalin, pro-opiomelanocortin, and prodynorphy. MENK and [Leu]-enkephalin are metabolized from proenkephalin and differ at the C-terminal amino acid, both acting in neurotransmission and pain treatment [1]. MENK, first discovered by Hughes in 1975 [2], is involved in growth regulation requiring receptor-peptide interaction [3–7]. MENK has also been renamed as OGF because of its growth regulatory activity other than as a neuro-modulator. OGF functions in development, cellular renewal, wound healing, angiogenesis, tumorigenesis and tumor progression [8–11]. As an autocrine and paracrine signal peptide encoded by the pre-proenkephalin A gene [12], the direct and rapid actions of OGF are not specific to a cell, tissue, or organ, but rather, are stereo-specific, reversible, non-cytotoxic, independent of serum, and occur at physiologically related concentrations [13]. Further, OGF is found in the cytoplasm of nearly all cells in culture and tumor explants.

1.2. Opioid growth factor receptor (OGFr)

The role of endogenous opioids as neurotransmitters is related to their binding to the opiate receptors [5,14]. In the treatment of addiction, enkephalins bind with the mu or delta receptors inhibiting the release of chemical substances, including substance P, vasopressin and dopamine. In regards to pain modulation, enkephalins and endorphins interact with mu and kappa opioid receptors. During the 1980s, a new opioid receptor was recognized and characterized in both normal brain and abnormal neural tissues [3,15]. This receptor was originally named zeta, to follow the naming principle of opioid receptors. Molecular studies suggest that the genomic and proteomic nature of the zeta receptor differs from the three classical opioid receptors (mu, delta, and kappa). The inhibitor properties of the zeta receptor on cell growth following binding with enkephalins, resulted in it being renamed opioid growth factor receptor (OGFr) [16,17], while MENK was renamed OGF. Subsequent studies found that the open reading frame for human OGFr was located on chromosome 20q13.3 [13] and was responsible for a 697 amino acid protein, with 8 imperfect repeats of 20 amino acids at the C terminus. Gene expression of OGFr has been detected in human fetal tissues including brain, lung, liver, kidney, and in adult heart, brain, liver, skeletal muscle, and pancreas. Expression of OGFr is located on or near the outer nuclear envelope and binds with OGF resulting in trans-locating inside the nucleus, and functions in growth regulation [18], not only in normal cells or tissues, but also cancer cells. Based on these observations, we hypothesize that the OGF-OGFr axis plays a role in development, cancer progression, cell renewal and

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homeostasis.

1.3. Opioid antagonists

NTX and naloxone are two opioid antagonists that are widely used in the treatment of drug overdoses, alcoholism and psychosomatic disorders. These antagonists were initially synthesized to prevent exogenous opiate interactions, and later used to isolate opioid receptors [19]. The structures of NTX and naloxone are similar: naloxone is n-allynoroxymorphone [20,21], while naltrexone is morphinan-6-1,17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxy-, hydrochloride [22]. Pharmacologically, naloxone is short-acting, whereas NTX is longer-acting and more potent. The two antagonists lack intrinsic biological activities on the classical receptors (mu, delta, and kappa) as well as OGFr [23,24].. Receptor blockage by these antagonists results in an increase in DNA synthesis, cell proliferation and full-thickness wounds healing [25]. Because the interaction between antagonists and receptors is reversible, the duration of opioid receptor blockade is responsible for the physiological outcome. Short-term exposure to NTX reduces cell growth, while long-term exposure results in growth acceleration [26–28]. In one study, it was suggested that low dosages of NTX can inhibit tumor growth, while high dosages of NTX resulted in responses that have little correlation with dosage but lead to larger tumors and accelerated mortality [29]. A murine model of neuroblastoma suggested that the growth-related properties of opioid antagonists are not dose-dependent, but dose-related and stereo-specific [30]. Further, the pharmacological properties of NTX and naloxone, coupled with the inability to regulate growth, have made them useful in the treatment of cancer, autoimmune diseases, and complications related to diabetes.

2. Mechanisms of OGF anti-tumor activity

OGF is a receptor (OGFr) mediated, pharmacophore with both activating and inhibitory activity, with no effect on tumor cell migration or invasion at concentrations ranging from 10^{-4} M to 10^{-6} M [31], and no cytotoxicity, apoptosis or necrosis, inducing properties even when concentrations of OGF increase beyond physiologic levels [32]. Extensive research has revealed the growth regulation of OGF and OGFr, especially on cancer modulation as shown in Fig. 1.

OGF binds to OGFr located on the nuclear envelope. The trafficking process into the nucleus of the complex is dependent on nuclear localization signals (NLS), of which NLS2 and NLS3 are essential. Karyopherin β plays an important role in the nucleocytoplasmic transportation. Being in the nucleus, OGF increases P16/P21 protein expression, and thereby delays the cell cycle transition from G0/G1 to S phase, with the results of cell proliferation decreasing. NTX can displace OGF bound to OGFr, causing a biofeedback production of more OGF and OGFr. The inhibitory effects of OGF-OGFr axis and/or NTX-OGFr axis can result in tumor repression.

2.1. Biological format of OGF and OGFr (OGF-OGFR axis)

Interactions between OGF and OGFr, inhibits cell growth, and influences homeostasis, re-epithelialization, tumorigenesis and tumor progression. Thus, the OGF-OGFr axis has an important role in maintaining epithelial homeostasis balance in the cornea [33], retina [34], and gastrointestinal systems. In addition, OGF and OGFr are expressed on the tongue epithelium, modulating cellular renewal in a circadian rhythm-dependent manner, demonstrating that DNA synthesis is circadian rhythm-dependent [35].

2.1.1. Up-regulation of OGF-OGFr axis

OGFr binding is increased by 2–8 fold, in cells from human squamous cell carcinoma of head and neck (SCCHN), when the receptor gene is over-expressed. Simultaneously, cellular proliferation is reduced 68% [36,37]. Over-expression of OGFr in an ovarian cancer cell line (SKOV-3) resulted in a significant decrease (36%–85%) in proliferation, and the cellular time significantly increased by 121% to 177% [38]. In the same research, it was proved that transplantation of cancer cells with OGFr over-expression into nude mice has a reduced tumor incidence, delayed tumor appearance, and decreased tumor volume [38]. Experiments to examine the mechanisms of these alternatives have focused on cell death, cell proliferation and tumor angiogenesis, suggesting that cell proliferation and vessel density were reduced 86% in mice inoculated with tumor cells over-expressing OGFr. However, necrosis or apoptosis is rarely found after exposure to OGF. Together, these results support that up-regulation of the OGF-OGFr axis by OGFr over-expression that can enhance cell growth inhibition.

2.1.2. Down-regulation of OGF-OGFr axis

Inhibition of OGF activity by silencing OGFr [39] in human ovarian cancer cell lines (OVCAR-3 and SKOV-3) revealed that cancer cells with low-expression of OGFr (OGFr siRNA) resulted in 70% less OGFr mRNA, 25% less OGFr protein, and a 31% increase in cellularity compared to controls. In contrast, the level of OGF between transfected cells and non-transfected cells was similar. In a large number of cancer studies, antisense cDNA and siRNA studies were shown to decrease expression of OGFr and reduce tumorigenesis in animal models [40], suggesting that down-regulation of the OGF-OGFr axis by OGFr could result in decreased modulation of axis activity on cell proliferation.

Together, these data suggest that attenuation of the OGF-OGFr system may exert an important influence on tumor growth in cancer patients.

2.2. Cell cycle regulation

OGF exposure can decrease the growth and increase the doubling time of cells, resulting in a significant increase in cells in the G0/G1 phase and a compensatory reduction in cells in the S and G2/M phase. Further, analysis showed a marked increase in the time of cells in the G0/G1 phase in some cancers. These results indicate that OGF binding is directed at the G0/G1 transition, supporting OGF inhibition of cell proliferation. DNA synthesis is also reduced by OGF, such that DNA synthesis, by colon cancer cells treated with OGF, had a significant decrease in the labeling index (LI) when examined at hours 1, 2, 4, 8 and 10; resulting in a reduction of 9% to 33% [41]. Meanwhile in the same studies, the percentage of mitotic cells exposed to OGF decreased by 24% to 48% at 1, 6, 8 and 10 h.

These studies also confirmed that the P16 and P21 pathway proteins that influence cell cycle were involved in the growth modulation by the OGF-OGFr axis. OGF binding to OGFr, delays transition of cells from the G0/G1 phase to the S phase by up-regulating cycling dependent inhibitory kinases such as CKI, P16 and P21. Protein expression of P16, a known tumor suppressor gene, is up-regulated by OGF in head and neck cancer cell lines, while the protein expression of P21 is increased in pancreatic carcinoma cells second to the mutation/deletion of P16 [42,43]. In normal human cells, the inhibitory action of OGF depends on both the $P16^{INK4a}$ and $P21^{WAF1/CIP1}$ signaling pathways. Expression of $P16^{INK4a}$ and $P21^{WAF1/CIP1,}$ have been evaluated in human umbilical vein endothelial cells, epidermal keratinocytes, dermal fibroblasts and mesenchymal stem cells, and shown to be significantly increased at different time-points by OGF with a similar impact on an increase in doubling time in studies using the cells mentioned above [44]. These studies demonstrate that cells transfected with $P16^{INK4a}$ siRNA, $P21^{WAF1/CIP1}$ siRNA or both $P16^{INK4a}$ and $P21^{WAF1/CIP1}$ siRNA are not inhibited by co-culture with OGF. This confirmed that the mechanism of OGF inhibitory activity requires the induction of P16 and/or P21 expression. In other studies, the expression of other cyclin dependent kinase inhibitors, including P15, P18, P19 and P27, had no significant alteration by OGF treatment in any cell line or tissue evaluated.

In other studies, the phosphorylation of retinoblastoma protein

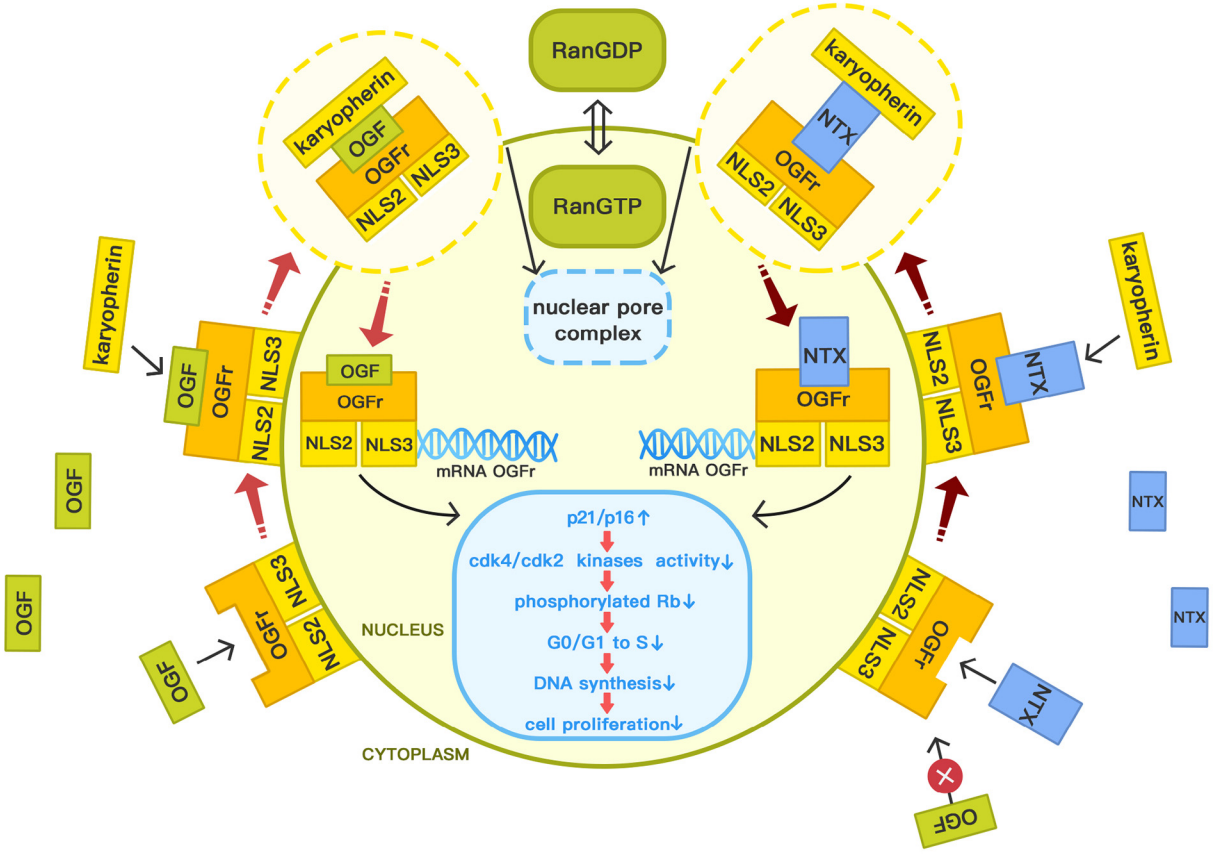

Fig. 1. Schematic diagram for OGF and NTX trafficking from cytoplasm into nucleus, and cell cycle regulation. OGF binds to OGFr located on the nuclear envelope. The trafficking process into nucleus of the complex is dependent on nuclear localization signals(NLS), of which NLS2 and NLS3 are essential. Karyopherin β plays an important role in the nucleocytoplasmic transportation. Being in the nucleus, OGF increases P16/P21 protein expression, and thereby delays the cell cycle transition from G0/G1 to S phase, with the result of cell proliferation decreasing. NTX can displace OGF bound to OGFr, causing a biofeedback production of more OGF and OGFr. The inhibitory effects of OGF-OGFr axis and/or NTX-OGFr axis can result in tumor repression.

(Rb), known to be necessary in the transition process from G to S phase was decreased by OGF while total retinoblastoma protein did not change [44]. It was observed that the phosphorylation of Rb down-regulated by OGF treatment was consistent with decreased CDK2 and CDK4 kinase activity. However, the total CDK2 or CDK4 protein levels were not reduced.

2.3. Translocation of the OGF-OGFr complex

A study to ascertain the cellular and sub-cellular locations of OGF and OGFr in the corneal epithelium, demonstrated that OGF and OGFr were co-localized in the para-nuclear cytoplasm and cell nuclei [18]. OGFr was detected on the outer nuclear envelope, in the para-nuclear cytoplasm proximal to the nuclear envelope, and perpendicular to the nuclear envelope in a nuclear pore complex. In summary, OGF interacts with OGFr at the outer nuclear envelope, and the complex translocates from the cytoplasm into the nucleus at the nuclear pore.

2.3.1. Temperature and clathrin dependence

Locations of OGF and OGFr expression have been identified in nearly all neoplastic cell studies, including cells in culture and tumor explants, as well as tissues derived from dermal derivatives. OGF binding with OGFr has been studied in two human cell lines, including a mesenchymal stem cell and a cancer cell line known to express both classical opioid receptor and OGFr. The results of this study showed that OGF internalization was a temperature and energy-dependent process [45]. In this study rhodamine-labeled OGF (RhoOGF) binding to African green monkey kidney cells (COS-7) was detected in the cytoplasm of cells incubated at 37 °C within 15 min, inside the nucleus after 30 min where it remained for 5 h. In contrast, there was no movement of RhoOGF when the cells were incubated at 4 °C. The study also proved that clathrin depletion significantly decreased the cellular entry of RhoOGF.

In another study, it was reported that transportation of the OGF-OGFr complex through nuclear pores and inhibition of cell proliferation required the nuclear localization signals (NLS). siRNA has confirmed that 2 of the 3 NLSs are essential for nuclear localization, including NLS383-386 and NLS456-460 [46].

2.3.2. Karyopherin β and ran dependence

Karyopherin β known to be one of the importins, and the RanGTP/RanGDP gradient across the nuclear envelope, have important roles in the nuclear cytoplasmic transportation of the OGF-OGFr complex [47]. Investigations have detected OGFr on the nuclear envelope where it binds with OGF. This complex associates with karyopherin, translocates through the nuclear pore, and is then observed in the inner nuclear matrix and at the periphery of hetero-chromatin of the nucleus. SiRNAs transfected to the karyopherin β1 or Ran results in a 44% increase in cellularity of human squamous cell carcinoma of head and neck cell lines (SCCHN). In contrast, there is no increase in cellularity when siRNA is transfected against karyopherin α1, α2, α3, α4 or α6. Several other key Ran interacting and regulating proteins, including nuclear transport factor 2 (NTF2), Ran binding protein 1 (RanBP1), Ran GTPase activating protein 1 (RanGAP1), and the nucleotide exchange factor RCC1, have been proven to be essential in mediating the second energy-dependent transport step of translocation of the OGF-OGFr complex

from cytoplasm into the nucleus.

2.3.3. CRM1 dependence for nuclear export

It has been revealed that the nuclear import of OGFr is important for its inhibitory function, while little is known about OGFr exporting from the nucleus. As we know, nuclear export is usually mediated by nuclear export signals (NES) recognized by eukaryotic protein known as chromosomal maintenance 1(CRM1) or exporting 1 [48], and Leptomycin B (LMB) can block CRM1-dependent nuclear export by binding to the NES binding domain of CRM1 [49]. In one study, the observation of significant nuclear accumulation of endogenous OGFr and OGFr-EGFP when exposed to LMB, demonstrated that OGFr was exported in a CRM1-dependent manner [50]. One consensus sequence for NES was identified in the same study. The NES was identified with leucines at residues 217, 220, 223 and 225. Leucine to alanine mutations of this NES resulted in decreased nuclear accumulation.

2.4. Activation of immune cells

The relationship between endocrine system and immune system was reported by Wybran et, al. in 1979 [51]. Following the findings, the opioid receptors (kappa, delta and mu) are detected on immune cells, such as macrophages, T cells, NK cells and dentritic cells. Many researches confirm that OGF is involved in the endocrine and immune system and could exert immune regulatory functions through binding to the OGFr receptors on immune cells. Studies have shown that OGF could activate immune cells directly or through the inhibition of regulatory T-cells. Immune systems can also be regulated by controlling the expressions of endocrine system signaling molecules. OGF binding to kappa and delta receptors on immune cells, rather than mu receptors, has also been found to influence immune regulation [13] as Fig. 2.

2.4.1. Effects on macrophage

Macrophages can be subset into two major types: The M1 phenotype and M2 phenotypes. The M1 phenotype is the classically activated macrophage, which responds to bacterial pathogens and inhibits tumor growth. In contrast, the M2 phenotype is the alternatively activated macrophage, which is induced in the presence of parasites and wounding, and can infiltrate tumors as tumor-associated macrophages (TAM). OGF can stimulate phagocytosis by peritoneal macrophages [52] resulting in the production of hydrogen peroxide and nitric oxide [53–55]. Studies have shown the regulation of macrophage types by OGF exposure which down regulate CD206 expression and the production of arginase-1, are markers of alternatively activated macrophages and tumor-associated macrophages *in vivo*. In contrast, CD64 and MHC-II expression and the induction of ROS, a marker of classically activated macrophages, are up-regulated in parallel. OGF can also increase the production of interleukin-12 and tumor necrosis factor-α, which have anti-tumor activities, while it decreases the secretion of interleukin-10 [56], an immuno-suppressive cytokine. In addition, OGF can increase the production of IL-1 and IL-6 [57,58] and induce M2 macrophage polarizing to an M1 macrophage phenotype, and modulate Th1 response.

2.4.2. Effect on T-cell function

It has been suggested that OGF can up-regulate T-cell rosettes on T-cells from lymphoma patients and healthy volunteers [59–61]. One study indicated that OGF secretion by colorectal cancer cells could inhibit both the number and function of T-cells [62], while another study reported that the OGF-OGFr axis could suppress T-cell proliferation [63]. OGF can increase the frequency of $CD8^+$ T cells, induce markers of T-cell activation and increase cytotoxic activity against mouse S180 tumor cells and secretion of IFN-γ [64]. Other studies have demonstrated that OGF can suppress the activity of $Foxp3^+$ Tregs, an important barrier to immunotherapy of cancer patients, and prevent

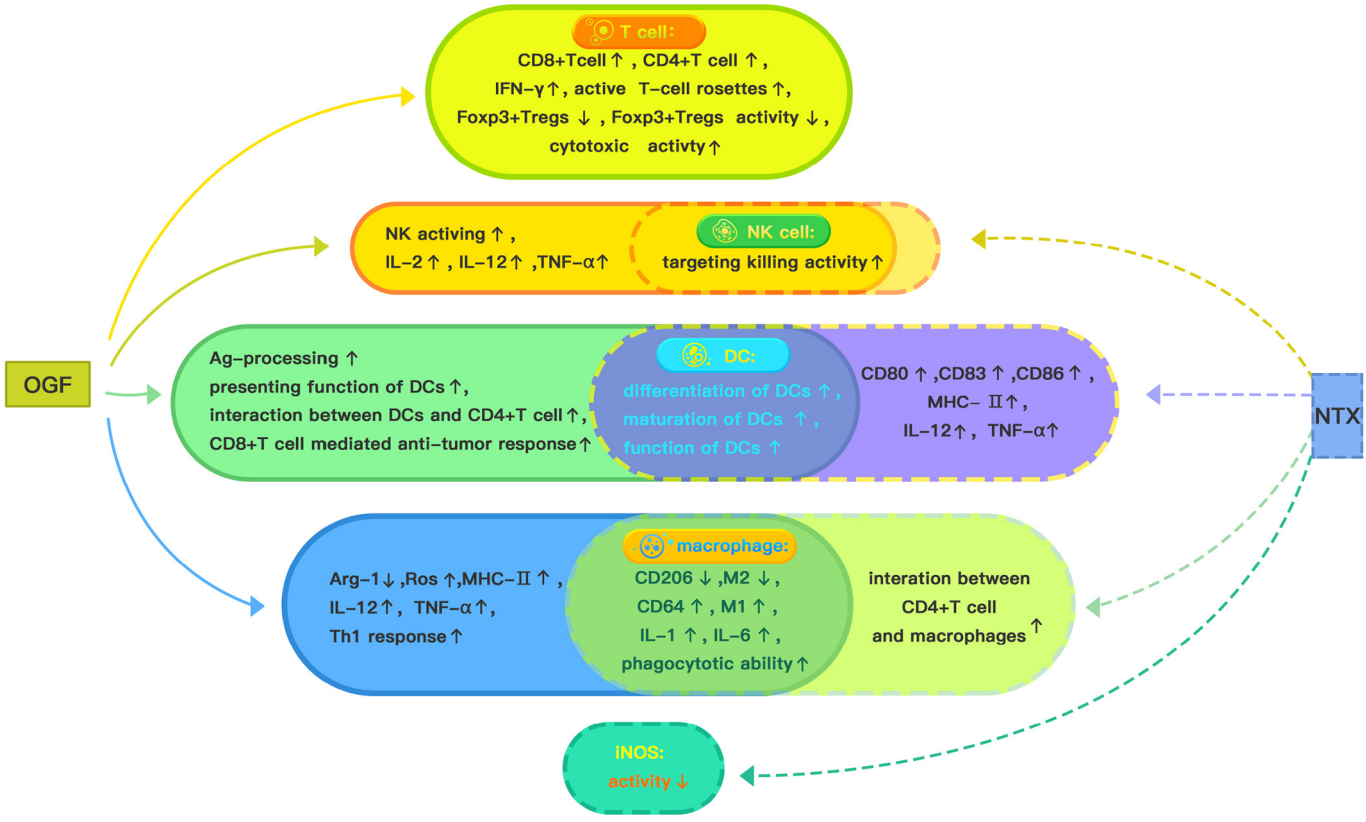

Fig. 2. Schematic diagram for anti-tumor immuno-regulation of OGF and NTX.

tumor progression by down-regulating Tregs [65]. OGF can also regulate lymphocyte subpopulations in the peripheral blood of cancer patients by inhibiting Tregs [66]. OGF can also up-regulate $CD4^+$ T cell expansion and CD4 molecule expression [67]. However, there remain questions about the effects of OGF exposure on T lymphocytes by OGF exposure.

2.4.3. Effects of OGF on NK cells

NK cells, a lymphocyte subset with the ability to secrete cytokines and kill tumor cells, are also stimulated by OGF, resulting in an inhibition of pulmonary metastases, as well as activation of NK cells [68]. Data support the role of OGF in the enhancement of NK cell activity [69–74] and in inducing production of IL-2, IL-12 and TNF-α [75–77]. Because NK cells have anti-tumor activity and can be regulated by OGF, this suggests another mechanism of action.

2.4.4. Effects on DCs

Previous studies confirmed that OGF could enhance antigen processing and presentation by dentritic cells (DCs) following binding of OGFr [78], which augments $CD8^+$ T cell mediated anti-tumor responses. OGF can also up-regulate interactions between DCs and $CD4^+$ T cells, increasing anti-tumor responses. In addition, OGF can induce differentiation, maturation and function of DCs, as well as DC polarization to an myeloid dentritic cell (mDC) subtype, and up-regulation of a type1 response [79].

2.5. Route of administration dependence

The inhibitory function of OGF for human SCCHN has shown that mice with xenografts of CAL-27 (a poorly differentiated SCCHN) given daily by intraperitoneal injections of 10 mg/kg OGF, had more than a 3-day delay in tumor development. Additionally, a decrease in tumor volume was noted, ranging from 51 to 64% in comparison to mice receiving OGF by continuous infusion by mini-pumps or by daily intratumoral injection [80].

2.5.1. Combination with immuno-modulators and chemotherapeutic agents

Data indicated that OGF combined with pidotimod (PTD), a synthetic dipeptide with immunomodulatory activities, could increase DC maturation [81]. Another study showed that imidazoquinolines, as immune response modifiers, can up-regulate OGFr, which in turn intensified the inhibitory action of OGF-OGFr axis on tumor cells [82].

A study designed to examine the additive effect of gemcitabine and OGF showed that tumor incidence, tumor volume, and latency time were increased in mice receiving combined therapy of gemcitabine and OGF. This indicates that the combination of OGF and gemcitabine results in growth inhibition greater than either agent alone [83]. In other studies, combination therapy with paclitaxel and OGF demonstrated that this combination of chemotherapy and biotherapy was more effective in inhibiting tumor growth than individual drugs and resulted in fewer side effects [84].

3. Mechanisms of anti-tumor action of NTX

NTX is a long-acting opioid antagonist that can block interactions between OGF and the 4 kinds of OGFr, and thus alters the OGF-OGFr axis. NTX can inhibit or increase cell proliferation as an immuno-regulator *in vivo* and *in vitro* within a specific concentration/dosage range. NTX is used for drug withdrawal and prevention of relapse at the dosage of 50 mg/day, and can be used to regulate chronic pain and treat immune diseases at the dosage of 5 mg/day, which is recognized as low dose naltrexone (LDN) [85]. It has been found that LDN can also regulate tumor cell proliferation through the OGF-OGFr axis [86,87] in the way of forming NTX-OGFr axis. (Fig. 1.)

3.1. Trafficking of NTX into cells and nucleus

Published studies have suggested that NTX could enter cells by passive diffusion rather than facilitated diffusion, and regulate DNA synthesis through displacement of OGF from its receptor (OGFr) on the outer nuclear envelope [88]. The energy-independent process was proven by studies on cells incubated in NTX at 37 °C and 4 °C. The results revealed that NTX, entering cells, was not dependent on pathways involving classic opioid receptors. NTX binding to OGFr, located on the cell nuclear envelope, would take the place of OGF, and then traffic into the nucleus in a way similar to that of OGF-OGFr complex.

3.2. Regulation of OGF-OGFr axis

It has been reported that intermittent blockage by LDN can result in a biofeedback production of more opioid peptides and receptors, and thus inhibit cell proliferation *via* compensatory up-regulation of OGF and OGFr. However, continued blockage can suppress the activity of OGFr. LDN can also reduce the tumor volume and delay the tumor development through the OGF-OGFr axis in SCCHN [89]. In OGFr-knockdown of human ovarian cancer cells, there is no inhibition of cell proliferation by LDN or OGF treatment [39], indicating that NTX inhibits cell growth by interacting with OGFr. These studies imply that the P16 and/or p21 cyclin-dependent inhibitory protein kinases may have an important role in the effects of LDN on cell proliferation and DNA synthesis [90–92]. This also demonstrates that the expression of pro-apoptotic proteins could be decreased by LDN through an activating apoptotic pathway [93]. In addition, the cells exposed to LDN become more sensitive to the chemotherapeutic drugs. LDN has also been reported to have more effective inhibition of tumor growth when combined with other agents such as aged garlic extract [94], vitamin D [95] and panobinostart [90].

3.3. Immuno-regulation and anti-tumor of NTX

Tumor growth can be reduced by regulating the function of the immune system by LDN. LDN can increase the phagocytic ability of macrophages by modulating the membrane marker expression (CD64 increasing and CD206 decreasing), and secretion of various cytokines (IL-1 and IL-6 increasing) [96]. LND can also increase the interactions between $CD4^+$ T cells and macrophages as evidenced by an increased concentration of IL-12 and tumor necrosis factor (TNF)-α [97]. Moreover, the expression of MHC-II, CD80, CD83, and CD86 on the membrane of bone marrow derived DCs (BMDCs) could be increased by LDN promoting the maturation and function of DCs. These studies reveal that the combination of LDN and OGF, as an anti-tumor therapy, could suppress DNA synthesis of pancreatic cancer cells and stimulate activation and proliferation of immune cells to promote the body to heal [98]. In addition, NTX can stimulate the cytotoxic activity of NK cells.

The mechanisms of LDN activity have not been fully understood; although recent studies suggest that LDN may function not only by specifically targeting the OGF-OGFr axis, but also through modulating the immune system. Further, activation of the apoptotic pathway by LDN can also relieve neuro-toxicity of glutamate on nerve cells by inhibiting the activity of inducible nitric oxide synthase (iNOS) (Fig. 2) [99].

4. Clinical application of OGF and LDN in cancer treatment

The clinical function of OGF and LDN was assessed several years ago in a phase I trial where late-stage cancer patients, who failed all standard therapy, had an improved life quality and prolonged survival time with OGF treatment [100]. A phase II clinical trial has also demonstrated clinical benefits to 53% of patients with intravenous infusions of 250 μg/kg OGF, compared with patients receiving gemcitabine or 5-fluorouracil [101]. In that trial, 24 subjects with advanced

Table 1
Cases of patients enrolled (N = 24).

	Number	%	
Age, years			
Mean ± SEM			61.6 ± 2.3
Range			46–80
Gender			
Male	12	50	
Female	12	50	
Stage of disease			
III	1	4	
IV	23	96	
Prior therapy			
Gemcitabine	23	96	
5-FU	4	16	
Prior surgery with recurrence	4	16	
Chemosensitizing radiation therapy	5	20	

Abbreviation: SEM, standard error for mean.

nonresectable pancreatic adenocarcinoma who failed standard chemotherapy were enrolled. Demographics of the study is shown in Table 1.

The median survival time for OGF-treated patients was demonstrated to be three times that of untreated patients (65.5 *versus* 21 days, $p < 0.001$), and quality of life surveys suggested improvement with OGF. Some other clinical trials of OGF treatment alone or in combination with other therapies, for various stages of hepatocellular carcinoma and head and neck cancers, have been undertaken resulting in improved outcomes [102,103].

A report by Berkson and his colleagues [104], showed that patients with pancreatic cancer achieved longer survival without any side effects when they were treated with the combination of LDN and α-lipoic acid (ALA/N). This protocol resulted in a decrease in tumor marker levels, improved parameters by physical examinations and reduced clinical symptoms. Two cases of children with congenital hepatoblastoma had improved disease free survival times of 10 years and 5 years after OGF/LDN treatment, suggesting LDN might be an alternative to conventional chemotherapy, with less toxicity [102]. Clinical trials of 10 patients with chemo-resistant metastatic cancer, demonstrated that the combined use of hydroxycitric acid (HCA) with α-lipoic acid (α-LA) and LDN, was safe and effective for treating refractory cancers in late stage, and this played an important role in modulating the cancer metabolism [105].

Together, these studies suggest that OGF and LDN hold promise as a biotherapy, not only in immune diseases, but also in malignant tumors.

5. Prospect

Clinical studies have demonstrated a role for OGF and LDN in regulating cell proliferation by both normal and cancer cells. These biological modulations suggest potential treatment *via* the regulation of the OGF-OGFr axis for cancers. This report summarizes the anti-tumor function of OGF and its antagonist (naltrexone), as well as the relationship between them. We posit that these drugs may have a useful mode of action or provide a new strategy for cancer therapy. One recent study reveals that OGF (MENK) could exert prophylactic and therapeutic influences in mice infected with A/PR/8/34 influenza virus [106], supporting that OGF can increase survival of mice infected with PR8 virus, decrease influenza replication, alleviate acute lung injury, and up-regulate opioid receptors (mu and delta). Thus, we may consider OGF to have adjacent activity for vaccine preparations, not only against influenza virus, but other tumor related viruses, such as human papillomavirus (HPV), Epstein-Barr (EB) virus and hepatitis virus. Although the anti-tumor mechanisms of OGF and its antagonist are not fully understood, therapy with OGF and/or LDN has advantages including safety, low toxicity, and ease of access. We submit that, in the future, this therapy will be an alternative for the treatment of human cancers.

Declaration of competing interest

All authors, formally inform the editorial office that we have no conflict of interest, and we will take legal responsibility if anything happens to this article.

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