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Review

Prospective oncotarget for gynecological cancer: Opioid growth factor (OGF) - opioid growth factor receptor (OGFr) axis

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ABSTRACT

The standard treatments for neoplasia include surgery, chemotherapy, hormone antagonists and radiotherapy, which can prolong survival, but rarely cure the tumors of gynecological cancer patients. OGF - OGFr expression, in various gynecologic cells and tissues, is an intersection point between cell development, neuroendocrine function and immune modulation. It has been identified that OGF and OGFr expression differs between gynecological tumor and normal cells. Further, exogenous or endogenous OGF and OGFr antagonists have been known to have a role in regulating cell viability and apoptosis. Moreover, the expression of proteins in the OGF - OGFr axis modulate differentiation and membrane expression of immune cells, which can enhance the immune response. In vivo and in vitro assays have shown that OGF and OGFr antagonists inhibit mitosis as well as induce apoptosis in gynecologic cancer cells. Although immune augmentation combination therapies can intensify cytotoxic activity, OGF or OGFr antagonists do not increase toxicities associated with dual-immune regulation. In conclusion, the OGF - OGFr axis provides significant strategies for antitumor efficiency in gynecological cancer.

1. Introduction

Globally, gynecological cancers have increased in incidence and mortality during the past decade [1]. Cervical cancer, uterine cancer and ovarian cancer form the most frequent gynecologic carcinomas and severely threaten the health of women. On an annual basis, these tumors include about 569,847 newly diagnosed and 311,365 deaths from cervical cancer, 382,069 newly diagnosed and 89,929 deaths from uterine cancer, and 295,414 newly diagnosed and 184,799 deaths from ovarian cancer, respectively [2]. Additional gynecological cancers include the less commonly occurring vaginal and vulvar cancers, and gestational trophoblastic neoplasia-like choriocarcinomas [3]. Despite traditional cytoreductive surgery, platinum-based chemotherapy and three-dimensional conformal or intensity-modulated radiotherapy provide the most common treatments for gynecologic tumors to delay tumor progression and improve quality of life. Without an efficient screening strategy or increased therapeutic activity, most of these patients are diagnosed at a terminal stage or are resistant to traditional therapeutics, which results in a poor prognosis [4–6]. The use of oncotargeted molecular therapies are expanding, including PD (programmed cell death protein)-1/PD-L1 monoclonal antibodies [7], Poly (ADP-Ribose) Polymerase inhibitor (PARPi) [8], as well as adoptive cellular immunotherapy, including chimeric antigen receptor (CAR) T-cells and dendritic cell (DC) vaccines [9], which have the potential to provide curative effect and prolong overall survival (OS). However, low levels of efficacy [10] and increased side effects [11] have restricted clinical applications supporting a need for additional research and development.

There is a linked relationship between tumor induction, disease progression, neuroendocrine interaction and immunity. It is generally acknowledged that neoplasms originate following genetic mutations, cellular damage and regeneration [12]; however, the tumor itself is rarely the direct cause of death. In the clinic, the majority of cancer patients die from the toxic side effects of chemotherapy, associated neutropenia, tumor related cachexia and corresponding complications [13,14]. The benefit patients derive from standard of care is limited and

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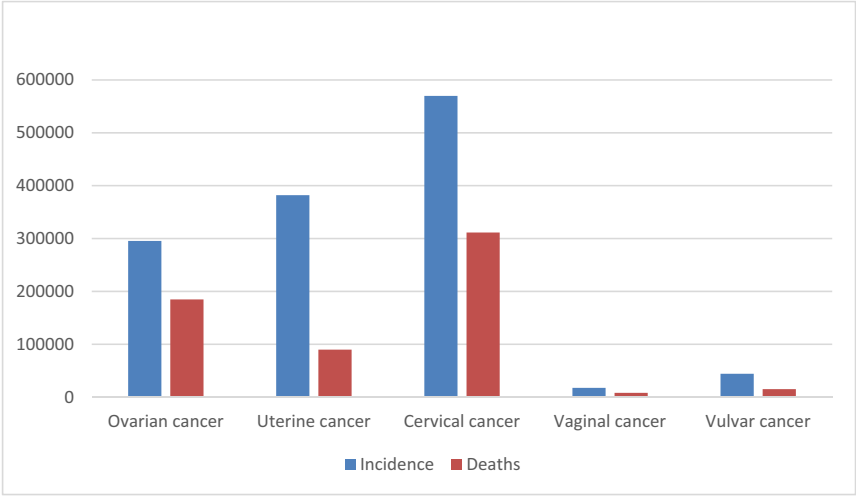

Fig. 1. General case of gynecological oncology.

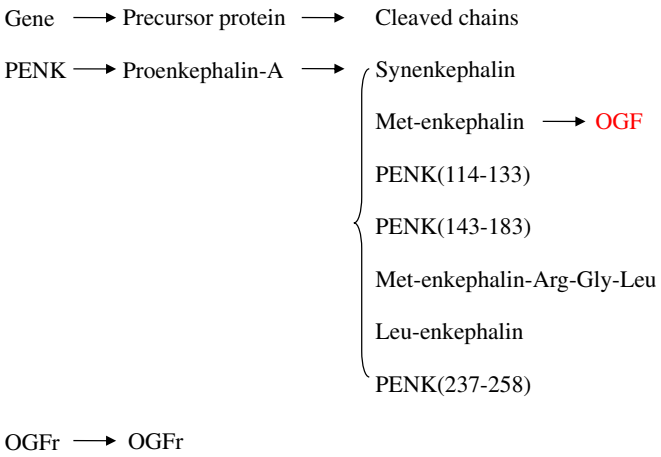

Fig. 2. Taxonomy of OGF and OGFr.

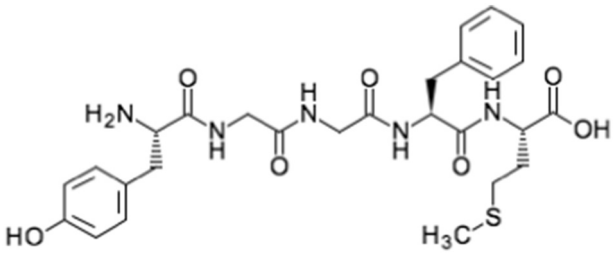

Fig. 3. Structure of OGF.

may reduce the quality of life. We have accepted the viewpoint that cancer is a chronic inflammatory disease, such that neuroendocrine and immunity regulates cancer occurrence and progression. In contrast, cancer may cause immunodeficiency and neuroendocrine and metabolic imbalances, leading to fatigue, anorexia and cachexia [15]. Consequently, understanding the interplay between the tumor, neuroendocrine interactions and immunity may provide clinicians an improved approach to control cancer and extend survival as opposed to traditional therapeutic strategies (Figs. 1–4).

2. OGF-OGFr axis

The endogenous opioid family is comprised of opioid peptides and opioid receptors that are ordinarily generated by the adrenal and

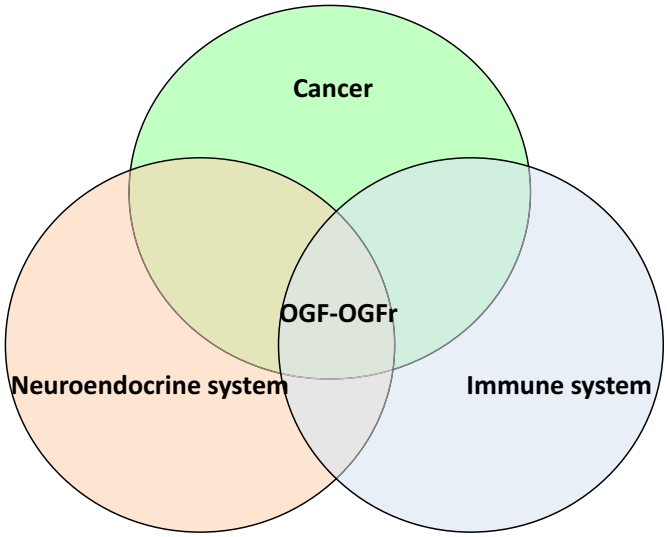

Fig. 4. The role of OGF - OGFr axis.

pituitary glands, and distributed through an autocrine or paracrine distribution pattern [16,17]. OGF, also termed methionine-enkephalin (MENK), is a native opiate pentapeptide derived from preproenkephalin and pro-opiomelanocortin genes, which is located at the 8q12.1 chromosome, and contains the structure H-Tyr-Gly-Phe-Met-OH [18,19]. As a natural ligand for OGFr, OGF provides a series of biological functions as a neuroprotective ingredient, including tissue development and organ maturation, DNA synthesis and cellular renewal, vascular generation and wound healing via binding to OGFr [20–22]. The OGFr is located at the 20q13.33 chromosome [23] and is characterized as a zeta-opioid receptor distributed to the cytoplasm and nucleus [24,25]. Three classic opiate receptors, mu-opioid receptor, delta-opioid receptor and kappa-opioid receptor contribute to the analgesic efficiency of opioids [26–28]. The OGF - OGFr axis is widely distributed on central and peripheral systems, especially the cerebrum and gastric-intestine (GI) tract [29]. OGFr is an integral membrane protein that is associated with the nucleus and following binding to OGF initiates a signaling cascade related to DNA synthesis and growth, and modulation of the immune and neuroendocrine systems following injury [23,30,31]. The action of OGF - OGFr is nonspecific, reversible, and dose-dependent, which is attributed to the duration of OGFr inhibition [32]. OGF or OGFr antagonists and low doses of naltrexone (LDN) have potential for anti-inflamation [28], antinociception [33], multiple sclerosis [34],

autoimmune encephalomyelitis [35], metabolism [36] and energy homeostasis [37], chronic hepatitis [38], anti-obesity and diabetic management [39].

3. OGF - OGFr axis and gynecological cancer

The OGF - OGFr axis has been identified in dozens of tumor cells, tissues and intraluminal secretions. Furthermore, natural or exogenous OGF depresses tumor proliferation by inducing cell cycle arrest and/or apoptosis while LDN inhibits neoplasm progression by blocking OGFr or up-regulating OGF - OGFr concentrations [40–44]. OGF and OGFr possess the property of dual-immune modulation for innate and adaptive competent cells and cytokines which could remodel tumor microenvironment (TME) by enhancing anti-tumor immunoactivity and remitting the immuno-suppressive state [45,46].

3.1. In vitro assay

OGF - OGFr are present in the cytoplasm and nucleocytoplasm of OVCAR-3, SKOV-3, SW626, CAOV-3, and HEY ovarian cancer cell lines and can block tumor growth in a dose-dependent way. A proliferation inhibition of 20% was observed with OVCAR-3 cells, following a 120 h co-culture and 23% on SKOV-3, 25% on SW626, 18% on CAOV-3, and 24% on HEY following 72 h of co-culture [47]. The antitumor effect was lost as OGFr was silenced, and further analysis showed p16 and/or p21 levels were up-regulated 4.1-fold and/or 2.3-fold after exposure to OGF for 9 h. In ovarian cancer cell lines SH-6 and SH-16 transfected with OGFr shRNA, cell numbers increased by 48–132% and 33–88% within 96 h without influencing the OGF level [48]. SH-6 and SH-16 cells with low OGFr expression had hyperactive proliferation as measured by BrdU incorporation; however, normal SH-6 and SH-16 clonal cells showed little apoptosis or necrosis. Similarly, proliferation of human squamous carcinoma cells, SCC-1 were depressed by 41% after exposure to OGF, whereas the cell number was increased by 54% after transfection with OGFr siRNA, and 42% after exposure to specific antibodies against OGF [43]. Thus, the OGF - OGFr axis may suppress tumor proliferation by stabilization of the cell cycle in the G0/G1 phase. The process is mediated by cyclin-dependent kinase inhibitory (CKI) pathways in which p16 and/or p21 are predominant and reduces phospho-Rb, which has a phosphorylated reaction in G1 phase via down-regulation of cyclin-dependent kinase protein (Cdk) 2 [49,50]. Other mechanisms include RNA and protein synthesis inhibition by an OGF or OGFr antagonist, in a non-specific, dose-dependent, reversible manner [51].

At present, our unpublished research data has revealed that human papillomavirus (HPV) ± human cervical cancer Hela (HPV 16+), Siha (HPV 18+), C33A (HPV -) cell lines have a depressed proliferation, but also induced apoptosis by co-culture with an exogenous OGF or OGFr antagonist (Fig. 6). Flow cytometry has verified the arrest of tumor cell mitosis and increased apoptosis by drug exposure for 48 h. Similarly, light microscopy, immunofluorescence and electron microscopy have revealed morphological pathologic, including irregular or rounder cells, lower cell density and adherence, intensive blebbing, hyperchromatic nuclei, excessive karyopyknosis and karyolysis in drug treatment group. Quantitative real time polymerase chain reaction (qRT-PCR) and Western blot analyses have confirmed cell-cycle related gene inhibition, as well as pro-apoptotic molecule expression, including caspase 3 and Bax activation. One mechanism of cellular apoptosis promotion by exogenous OGF may be the activity of the PI3K/AKT/mTOR pathway through OGFr [52,53]. Intermittent or short exposure to an OGFr antagonist would boost OGF presentation in the tissue which reacts analogously with OGF on tumor proliferation modulation [54]. In addition, OGFr antagonist transduces extracellular signals to the cytoplasm and nucleus via regulating extracellular regulated kinase (ERK), Ca^{2+} channels and synergistically activating protein kinase C (PKC), increases integrin α7 level, and therefore influences tumor cells malignant biological behavior [55–57].

3.2. In vivo assay

Mice with a xenograft of the human ovarian cancer cell line SKOV-3 had an association with the expression of OGF and OGFr in the cytoplasm and nucleus of tumor tissue. Further, the severity of disease progression was negatively correlated with OGF and OGFr concentrations [58]. In a female mouse model, the number of intraperitoneal metastatic nodes of SKOV-3 ovarian carcinoma were reduced by 65% with a dosage of 10 mg/mL OGF given by intraperitoneal injection and 51% with a dosage of 0.1 mg/kg LDN. OGFr expression was reduced by 29% in the OGF administration group and increased by 63% following LDN administration group. To assess the mechanism of tumor growth retardation, tumor cell BrdU labeling was found to be decreased 61% with exposure to OGF and 52% with exposure to LDN. Furthermore, the vascular density of tumor tissue with OGF and LDN treatment was reduced 89% and 73%, respectively. Established mouse models with under-expression of OGFr have a delayed tumor induction in the low OGFr group that was 21–22 days and 7–14 days shorter with 1–2 × 10^6 SH-6 and SH-16 cells [48]. Following injection with 10 mg/kg OGF, there was a 100% macroscopic SH-6 tumor incidence in the under-expressing OGFr group by day 7, in the WT group by day 19 and in the EV group by day 21, respectively. Tumor volumes were enlarged by 41–267%, 4–14 days later comparative to controls. Exposure to OGF in WT and EV groups showed notable tumor protective effects including a decrease of 38–60% in volume and 32–55% in tumor weights relative to saline, which suggests that OGFr stimulated tumor development and OGF slowed tumor growth by binding to OGFr.

Tissue specimens from ovarian sites and omental metastases in 53 terminal ovarian cancer patients, and a comparable ovarian cyst were assessed by Fanner, et al., using semi-quantitative immunohistochemistry [59]. In these studies, the distribution of OGF and OGFr in cytoplasm and nucleus was similar among ovarian cancer, ovarian cyst and HOSE cells. However, the expression of OGF was reduced by 29% in ovarian cyst and 58% in ovarian cancer cells, and the expression of OGFr decreased 34% in ovarian cyst and 48% in ovarian cancer cells, respectively. Although OGF exhibited a specific binding to nuclear OGFr, the binding capacity in ovarian cancer was 81% lower compared with ovarian cyst without OGF - OGFr affinity discrepancy.

OGF and OGFr have also been proven to occur on human squamous epithelium and endometrium [60]. In the tissue of human squamous cell carcinoma, OGFr levels were down-regulated by 79% compared with that in normal tissue, and 42% compared with that in para-cancerous tissue [61]. The binding ability to nuclear OGFr of OGF had a nine-fold reduction in comparison with that of normal tissue in which OGFr was evenly expressed. Nonetheless, OGFr mRNA displayed no marked variation among epithelial mucosa, tumor margin and tumor tissue, prompting OGFr protein reduction to be triggered in the process of post-transcription. Studies of uterine membranes revealed that OGF accelerates cellular apoptosis via the Fas/FasL and/or NFAT1 signal pathway [62,63]. In addition, except for this antineoplastic mechanism of decreased cellular Ca^{2+} uptake, OGF and estradiol hormone, which are co-factors to promote cell growth and tissue development, and may also play a significant role in hormone-dependent uterine carcinoma [64,65].

3.3. Immuno-regulation

In view of immune modulation by opioids via opioid receptors [66,67], investigators have explored the role of the OGF - OGFr axis on the immune system. Generally speaking, the function of the OGF - OGFr axis is more like a double-edged sword. On the one side, OGF and OGFr antagonists suppress immune reactivity, providing a functional remedy for autoimmune disease [32,68]. On the other side, these two molecules could enhance the antineoplastic response of immune cells and

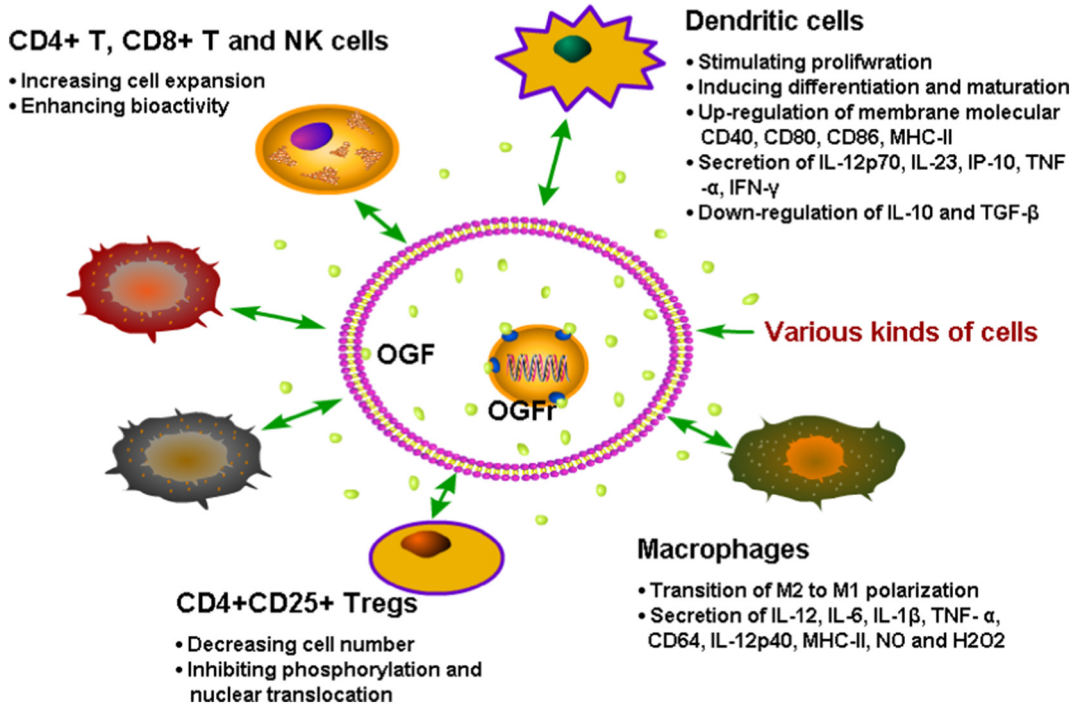

Fig. 5. Diagrammatical illustration of OGF - OGFr axis.

molecules [52,69]. The OGF - OGFr axis acts on toll-like receptors (TLRs) that are vital in initiating innate and adaptive immunoactivities to defeat inflammation and neoplasm [70–72]. TLRs belong to the family of pattern-recognition receptors and activate cytoplasmic downstream signal pathways to regulate released cytokines and chemokines [73]. However, dysfunctions are induced during induction and development, resulting in immunologic tolerance, which is closely related to the TME [74]. The professional antigen presenting cells (APCs), dendritic cells (DCs) can be stimulated to proliferation, differentiation and maturation by OGF and OGFr antagonist (Fig. 5), with larger cell bodies, more pseudopods, and up-regulation of membrane molecular CD40, CD80, CD86, MHC-II and secretion of IL-12p70, IL-23, IP-10, TNF-α, IFN-γ while down-regulation of IL-10 and TGF-β [46,75,76]. One mechanism of OGF regulation of DC function is via the TLR4/MyD88/NF-κB molecular pathway [46]. OGF and OGFr antagonists can promote tumor association macrophages (TAMs) transition from M2 to M1 and production of IL-12, IL-6, IL-1β, TNF-α, CD64, IL-12p40, MHC-II, NO and H_2O_2 [52,77,78]. In addition, OGF significantly activates lymphocyte subtypes of CD4 + T cells, CD8 + T cells (CTL) and natural killer cells (NK) while depressing negatively CD4 + CD25+ Tregs [79]. OGF decreases the number of CD4 + CD25+ Tregs as well as phosphorylation and nuclear translocation of Smad2/3 that participates in the TGF-β pathway [80]. In regard to CTL, OGF matures phenotype and competence via increasing FasL, CD28, Prf levels and cytotoxicity [81].

3.4. Additive antitumor potency

To compare the oncotherapeutic effect of OGFr antagonists, researchers have cultured SKOV-3 tumor with LDN, cisplatin, taxol and LDN plus cisplatin or taxol, respectively [82]. The number of tumor cells with LDN (10^{-5} mol/L) and taxol (10^{-10} mol/L) decreased by 61% and 31%, compared with LDN plus taxol (Fig. 7), respectively, while combinations of the two had identical tumor inhibition efficacy related to higher concentrations of taxol (10^{-9} mol/L). The number of cells within the cultures with LDN plus cisplatin were reduced 42% and 32% in contrast with LDN and cisplatin, respectively. A reduction in tumor duration has been observed in mice with subcutaneous transplantated SKOV-3 tumor that was 21–48% with LND, 21–54% with

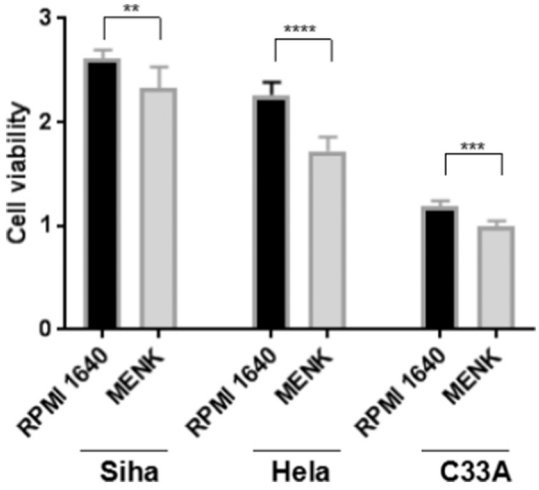

Fig. 6. Cell viability of cervical cancer cell lines after OGF exposure by MTS.

taxol, 24–54% with cisplatin, 39–60% with LDN plus taxol, and 16–60% with LDN and cisplatin, respectively. In addition, vascular density was depressed 52–73%, compared in single or combined reagent administration. OGFr presentation increased 46–61% in animals given LDN alone and PDL plus taxol or cisplatin. In clinical trials, patients who experienced chemotherapy failure could gain varying degrees of benefit ranging from delayed development to complete remission by LDN and chemotherapeutics [83–86]. At present, we suggest that OGF enhances the antitumor effect of molecular oncotargeting medicines in ovarian and cervical cancer cell lines. We conclude that there is clinical value via antitumor activity and reduced toxicities occur without affecting antitumor effect.

4. Prospection

The OGF - OGFr axis is involved in biochemical/hormonal reactions and in the maintenance of homeostasis. In the face of neoantigens or

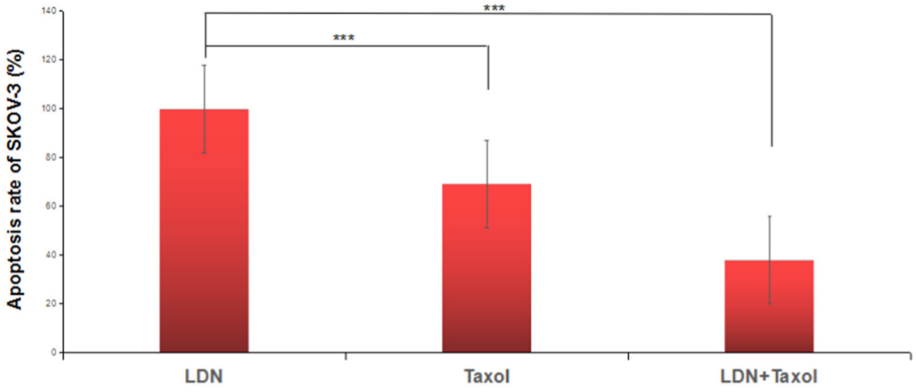

Fig. 7. The tumoricidal efficiency of LDN (10^{-6} mol/L) and taxol in vitro.

tissue toxicity, OGF or OGFr antagonist can activate natural immunity and specific killing to delete detrimental responses, while it averts an inflammatory over-response that may evoke further damage by immune cells and molecules via inhibitory cell multiplication or accelerated apoptosis, co-efficiently with neuro and endocrine system. Opioids have long been used for analgesia and chronic pain [87]. Limited by the low survival rate of traditional surgery, chemo- and radio-therapy against advanced cancers, intensive studies on immunologic escape and the TME search for a more suitable regimen for improving outcome [88]. There is increasing evidence that support an increase in associated toxicities with standard therapy [89]. Until now, the veil of OGF - OGFr axis on antitumor competence has been obscure, accounting for an essential part of immune modulation and tumoricidal activity. Future studies need to probe into tumor cytotoxicity and evaluate clinical effects of exogenous OGF and OGFr antagonists in gynecological cancer.

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