Targeting the opioid growth factor: Opioid growth factor receptor axis for treatment of human ovarian cancer

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Abstract

The opioid growth factor (OGF) – opioid growth factor receptor (OGFr) axis is a biological pathway that is present in human ovarian cancer cells and tissues. OGF, chemically termed [Met^5]-enkephalin, is an endogenous opioid peptide that interfaces with OGFr to delay cells moving through the cell cycle by upregulation of cyclin-dependent inhibitory kinase pathways. OGF inhibitory activity is dose dependent, receptor mediated, reversible, protein and RNA dependent, but not related to apoptosis or necrosis. The OGF-OGFr axis can be targeted for treatment of human ovarian cancer by (i) administration of exogenous OGF, (ii) genetic manipulation to over-express OGFr and (iii) use of low dosages of naltrexone, an opioid antagonist, which stimulates production of OGF and OGFr for subsequent interaction following blockade of the receptor. The OGF-OGFr axis may be a feasible target for treatment of cancer of the ovary (i) in a prophylactic fashion, (ii) following cytoreduction or (iii) in conjunction with standard chemotherapy for additive effectiveness. In summary, preclinical data support the transition of these novel therapies for treatment of human ovarian cancer from the bench to bedside to provide additional targets for treatment of this devastating disease.

Keywords: OGF, OGFr, [Met^5]-enkephalin, naltrexone, cell proliferation, DNA synthesis, ovarian cancer

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Introduction

Ovarian cancer

Malignancies of the ovary are derived from germ cells in the endoderm, stromal tumours from connective tissue and epithelial cells that provide a covering for the ovary and Mullerian ducts. Epithelial-derived tumours representing approximately 90% of all tumours of the ovary generally arise in the fourth decade of a women's life and are usually malignant.[1,2] Because markers for ovarian cancer are limited in their effectiveness, the diagnosis is often delayed until the disease is at an advanced stage and consequently is associated with a high mortality rate. Ovarian cancers can be classified as either type I or II.[3,4] Type I which are low grade, resistant to chemotherapy, but responsive to hormonal treatment, and are usually diagnosed at an early stage. Type II cancers are more prevalent, are serous cell in origin or undifferentiated, grow aggressively and respond to chemotherapy if detected early. Ovarian cancer is responsible for nearly 3% of all cancers in women and is the fifth leading cause of death among women in the USA.[5] Ovarian cancer is the leading cause of death from gynaecological malignancies in the USA, with approximately 15 out of 1000 women developing cancer of the ovary at some time in their life. In the USA, the median age at death from ovarian cancer is 71 years, and Caucasian women have a higher death rate than Blacks, Hispanics or Asians.[6] Despite a decline in the incidence of ovarian cancer worldwide, there are an estimated 140,000 deaths worldwide each year from this cancer; less than 40% of all women survive longer than five years.[7] Prevalence of cancer of the ovary in 2009 was reported to be 183,000 women in USA.[7]

Ovarian cancer is multifactorial with regard to its risk factors and origin. Age, genetics and a familial history of cancer increase the risk; frequently, cancer of the ovary occurs in patients or families with a high incidence of breast and/or colon cancer.[8–10] The highest risk for ovarian cancer appears to be family relationships with a similar disease.[11] Genetically, linkage between the BRCA1 locus on chromosome 17q21and BRCA2 locus on chromosome 13q12[12] has suggested that mutations in either of these genes results in increased risk of developing ovarian cancer,[12,13] but women with germline mutations in either gene are more likely to survive five years after diagnosis relative to women who do not have such mutations.[1,13] In families presenting with several generations of ovarian cancer, prophylactic oophorectomy is recommended. Mutations in BRCA1 genes and loss of OGFr expression would both result in enhanced ovarian cancer growth, but

research on interfacing between BRCA genes and the OGFr gene is unknown.

Younger age, lower stage of tumour at initial identification, well-differentiated tumours and the absence of ascites in individuals with type I ovarian cancer are supportive of a good prognosis following initial detection of ovarian tumours. Patients who progress to having stage III and stage IV disease are usually reliant on several rounds of chemotherapy. Even removal of the cancer mass does not reduce mortality rates as the disorder has a recurrence rate of 40–50% within three years.[14] The five-year survival rate remains as low as 10% even following combination therapies. Thus, there is a medical need to continue to explore new approaches for treatment of ovarian cancer.

Current Treatments

The National Cancer Institute reports that more than $5 billion are spent annually in USA for ovarian cancer treatment. Surgery remains the first choice in treatment, but complete cytoreduction is not always an option. Following cytoreduction, or in cases where recurrent disease occurs, the primary choice of adjuvant chemotherapy is combination carboplatin and paclitaxel. Both agents are well known pro-apoptotic chemicals that destabilize DNA or interfere with microtubule assembly and are associated with substantial side effects that often reduces the ability of patients to tolerate a full round of treatment. Recurrent ovarian cancer is usually treated with additional chemotherapies such as gemcitabine or doxorubicin, and the dose and treatment regimen is dependent on how rapidly the disease recurs. A reappearance of disease suggests that the cancer is resistant to one or more of the treatments.

Thus, our knowledge about the biology of cancers of the ovary supports the need for new and novel therapies that target the biology of the cancer. These therapies may be used in conjunction with surgery as adjuvant therapy, as preventative therapy targeting cell proliferation following surgery or to increase sensitivity to standard therapy when patients demonstrate increased resistance to chemotherapy.

OGF-OGFr Axis

The opioid growth factor (OGF)–opioid growth factor receptor (OGFr) axis was originally identified and characterized more than three decades ago. The pentapeptide OGF is chemically termed [Met^5]-enkephalin and was identified as a growth-modulating agent in mouse neuroblastoma cells and developing rat brain.[15–18] Subsequent studies have shown the peptide to be autocrine and paracrine produced in tissues from all three dermal derivatives.[19,20] OGF has been identified in nearly all tissues of the body with varying levels of expression. Brain tissue has one of the highest levels of enkephalins followed by intestinal organs.[20,21] The function of growth inhibition being associated with OGF was first reported in 1987 using developing rat brain[17] and mouse neuroblastoma cell lines[18]. Since then, OGF has been shown to have inhibitory effects on normal cells and tissues,[22–24] neoplasia[25–33] and bacteria.[34] OGF is present in developing and renewing tissues,[20,35,36] its action is dose related, time dependent, receptor mediated and reversible.[22,23,28–33] OGF is metabolized quickly in tissues and plasma and exists in human plasma for less than 20 min after secretion.[37]

Early studies to identify the receptor involved in growth emanated from work whereby the opioid antagonist naltrexone was administered to animals at different dosages.[38–45] High dosages of naltrexone blocked opioid receptors for extended periods of time and resulted in accelerated growth of body,[38] organs[40] and brain[38,41] in rat. Further analyses using radio-labelled enkephalins demonstrated that the receptor associated with growth was novel.[46–50] The receptor was originally termed zeta (ζ) to reflect its opioid receptor characteristics of being blocked by opioid antagonists such as naloxone and naltrexone. However, further studies revealed that the receptor was substantially different from other classical opioid receptors and was renamed OGFr. OGFr is nuclear associated, rather than being cytoplasmic in distribution, and shares little to no homology to the mu, delta or kappa opioid receptors at both molecular and protein levels.[51–54]

OGF binding to OGFr is specific and saturable and has a one-site model of kinetics. Biochemical [46–49] and morphological[55–57] studies have shown that OGFr is associated with the nucleus. Subcellular fractionation studies revealed that OGFr is an integral membrane protein associated with the nucleus[47–50] often located on the outer nuclear membrane.[55,56] OGFr has been cloned in mouse,[53] rat[51] and human,[52] and the human chromosomal identification is 20q13.3.[54] Of interest, this chromosomal region is often amplified in ovarian cancer.[58] At the molecular level, OGFr has three bipartite nuclear localization signals,[57] facilitating transport into the nucleus where immunoelectron microscopic studies have detected OGF interacting with OGFr.

Receptor knockdown studies using siRNA technology demonstrate that the regulation of cell proliferation by the OGF-OGFr axis appears to be dependent on nucleocytoplasmic transport by karyopherin β1 as well as the RanGTP/RanGDP gradient across the nuclear envelope.[57] In an extensive screening, 31 different human cancer cell lines were evaluated for the presence and function of OGFr, and human ovarian cancer cell lines SKOV-3 and OVCAR-3 were reported to have both receptor and peptide.[33]

Presence and function of the OGF-OGFr axis in human ovarian cancer cell lines

The presence of the OGF-OGFr axis has been documented in a number of human cancers including neuroblastoma,[18,27,45] pancreatic,[26,59] colon,[25] renal,[29] squamous cell carcinoma of the head and neck,[60–62] hepatocellular adenoma[31] and breast.[32] *In vitro* and *in vivo* studies revealed that OGF inhibited cell proliferation in culture[25–29] and when transplanted into nude mice.[18,39,42,59–61] However, the question remained as to whether the OGF-OGFr axis was functional in human ovarian cancers. Utilizing two human ovarian cancer cell lines that represented epithelial-derived ovarian neoplasms, the presence of OGF and OGFr were

detected by immunocytochemistry in the nucleus and cytoplasm of each cell line.[58] OGFr was quantitated by binding assays using [^{3}H]-[Met^5]-enkephalin. Specific and saturable binding was reported in both cell lines, with binding capacities of 4.5 fmol/mg protein or less being detected; binding affinities were ~5 nM.

Functionally, the addition of exogenous OGF was shown to suppress growth of human ovarian cancer cells in a dose-related manner over a period of 120 h; inhibitory effects were noted within 24–48 h.[63] OGF treatment at 10^{-6} M over a period of five days reduced cell number of OVCAR-3 cultures by as much as 27% and SKOV-3 cultures by as much as 23%; higher concentrations of OGF reduced growth up to 51% relative to sterile water-treated ovarian cancer cell cultures. OGF's inhibitory action was opioid-receptor mediated, and the receptor was identified as OGFr, and not mu, delta or kappa opioid receptors. The repressive activity of OGF was reversible, and dependent on RNA and protein synthesis as determined by addition of puromycin, cycloheximide or actinomycin D at concentrations that did not alter growth themselves. Examination of the mechanism behind the reductions in cell number revealed that OGF had no effect on apoptosis or necrosis but did target the cell cycle. Specifically in these cell lines, OGF upregulated p16 cyclin-dependent inhibitory kinase protein in synchronized populations for OVCAR-3 cells by 2.6-fold within 1 h of OGF treatment and 4.1-fold after 9 h of OGF exposure relative to levels of p16 in vehicle-treated cultures. Protein expression of p21, another cyclin-dependent inhibitory kinase, was upregulated by 2.8-fold over controls within 5 h of OGF exposure. Knockdown of the p16 and p21 proteins using siRNA technology revealed that either p16 and/or p21 protein induction is required for the inhibitory action of OGF, and thus repression of cell movement from the G_1 to S.[58]

OGF-OGFr inhibition of human ovarian cancer cells transplanted in nude mice

To address the question of whether OGF could effectively modulate growth of human ovarian cancer cells transplanted into nude mice, studies were conducted to measure tumour volume in mice with human ovarian cancer cells transplanted either subcutaneously or intraperitoneally and once tumours were visible, treated with OGF and/or with one of the standard chemotherapies (i.e. paclitaxel or cisplatin).[63,64] These studies addressed whether OGF provided a protective effect for mice receiving the well-known toxic chemicals paclitaxel or cisplatin, and whether the effectiveness of the standard chemotherapy to inhibit tumour growth could be enhanced by the addition of OGF. Combination treatment of SKOV-3 cells *in vitro* with OGF (10^{-6} M) and either taxol (10^{-9}or 10^{-10} M) or cisplatin (0.01 or 0.001 μg/ml) reduced cell number and DNA synthesis to an extent greater than that recorded for any individual compound. OGF, but not taxol or cisplatin, altered growth in a receptor-mediated manner, and growth was reversible with the withdrawal of OGF; cell cultures treated with taxol or cisplatin showed significant levels of apoptosis or necrosis and growth inhibition was not reversible.

To answer the question whether OGF could modulate growth of ovarian cancer *in vivo*, female nude mice were inoculated with SKOV-3 cells and treated daily with OGF (10 mg/kg) for 5 weeks; OGF reduced tumour volume by as much as 50% relative to tumour measurements in mice receiving saline.[63] Injections of cisplatin or taxol into mice with SKOV-3 tumours reduced tumour volume to a level comparable to OGF, but the combination of OGF to each agent resulted in an additive inhibitory effect (Figure 1). Beginning on day 2 following subcutaneous xenografting of human ovarian cells, tumour volumes were reduced by OGF (26–50%), taxol (22–50%), cisplatin (31–58%),

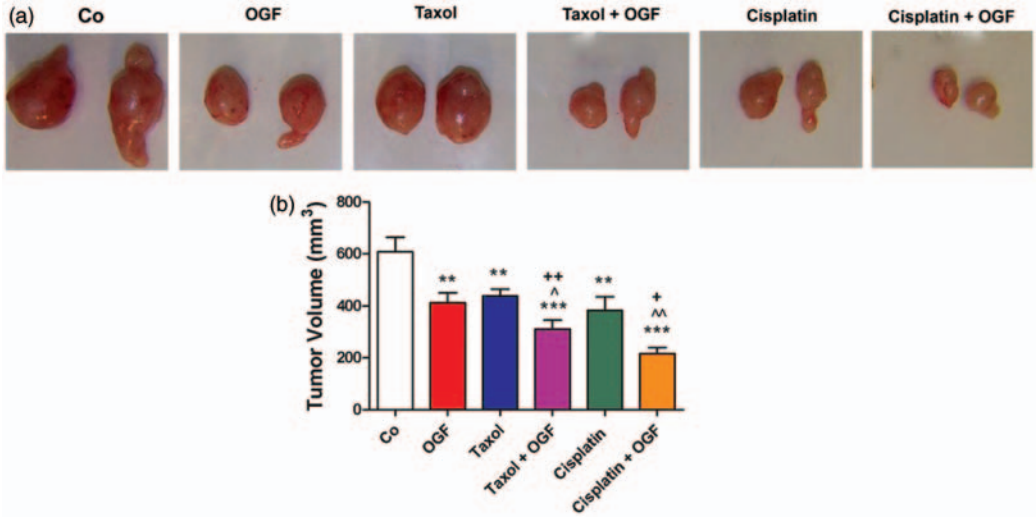

Figure 1 Representative images (a) of SKOV-3 ovarian tumours subcutaneously xenografted into female nude mice and treated with OGF, paclitaxel (taxol), cisplatin, or a combination. At the time tumours were visible, mice were injected with OGF (10 mg/kg, daily), taxol (3 mg/kg, once weekly for 5 weeks), cisplatin (4 mg/kg, days 0 and 7), taxol and OGF, cisplatin and OGF, or an equivalent volume of sterile saline (Co). (b) Tumour volume measured on day 37. Values represent means ± SE for 12 mice/group. Significantly different from saline controls at $^{**}P < 0.01$ and $^{***}P < 0.001$, from OGF at $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ and from taxol or cisplatin alone at $^{+}P < 0.05$ and $^{++}P < 0.01$. Adapted from Figure 3 in Ref. 63. (A color version of this figure is available in the online journal)

OGF + taxol (21–62%) and OGF + cisplatin (30–70%) relative to the tumour volume in saline-injected control mice. Furthermore, the addition of OGF to the regimens or cisplatin or taxol provided some protective effect because body weight loss, normally associated with cisplatin or taxol treatment, was diminished in mice receiving the combined therapy. Evaluation of the tumours for underlying mechanisms associated with the tumour size reductions showed that the combination therapy of OGF plus cisplatin or taxol has an additive inhibitory effect on DNA synthesis and angiogenesis relative to individual treatments or saline. These preclinical data in mice revealed the feasibility of using OGF as a non-toxic therapy, either alone or in combination with standard of care therapy. Moreover, OGF seems to provide some protection against the systemic toxicity and body weight loss that occurs following consumption of taxol or cisplatin alone. Finally, OGF might be warranted as a therapy for treatment of patients following surgical reduction of ovarian tumours, as the tumour burden would be significantly reduced, and OGF would be effectively able to manage any residual neoplastic cells.

Genetic modifications of OGFr: Over-expression and under-expression of receptor

Many of the cellular and molecular events involved in cancer of the ovary are unknown, and the pathogenesis of this disease needs to be evaluated to define improvements in treatment. Given our knowledge that the OGF-OGFr axis is present in human ovarian cancer cell lines[58]and tumours,[63] and data from screening human cancer cells,[33] we investigated whether OGFr plays an integral role in the biology of human ovarian cancer. To examine how genetic manipulation of the OGFr receptor may influence tumour progression, we established cell cultures of SKOV-3 human ovarian cancer that stably over-expressed[65] or under-expressed[66] OGFr. Cultures were transfected with either pcDNA3.1 + vector (empty vector group) or with plasmids containing pcDNA3.1 + human OGFr, and cells were selected and amplified. Over-expression of OGFr upregulated receptor number (i.e. binding capacity) 51–154% relative to Bmax values in wild-type or empty vector transfected SKOV-3 human ovarian cancer cells; binding affinity was comparable among all clonal lines and wild-type cells.[65] Cell growth *in vitro* was significantly decreased (up to 85%) over a five-day period of time relative to wild-type cells, and doubling times for the transfected cells were increased as much as 177% relative to controls. These data demonstrate a delayed proliferation in clonal lines expressing more receptor and responding to inhibitory endogenous enkephalins. Clonal lines over-expressing OGFr were treated with exogenous OGF and cell growth was reduced 70–85% in comparison with wild-type cells treated with this peptide. Summation of the basal growth inhibition observed and the inhibition induced by exogenous OGF revealed that growth was inhibited 2.5- to 4.6-fold over wild-type cells across the five-day period of time.

To inquire whether molecular manipulation of OGFr alters tumourigenesis, two clonal lines of human SKOV-3 ovarian cancer cells over-expressing OGFr that were characterized *in vitro* to inhibit growth and over-express OGFr were transplanted into female nude mice; tumour cells transfected with empty vectors and wild-type cancer cells were also xenografted into mice.[65] Within 4–5 days of injection of cells, wild-type and empty vector tumours were measurable in 90–100% of mice, whereas no mouse receiving the ovarian tumour cells over-expressing OGFr had a measurable tumour. This suggests that the endogenous enkephalins were capable of delaying tumour growth. Approximately three weeks later, not all mice receiving cells over-expressing OGFr had measurable tumours, and at the end of the study (day 32), 60% of mice receiving clonal line OGFr-3 did not develop a tumour. Latency time from cell inoculation to a measurable tumour (i.e. 5 mm diameter) were 12 days longer in the OGFr-3 clone, and 4 days longer in the OGFr-22 clonal line, than the latency for mice receiving wild-type cells. Tumour volumes over the 5.5 week period of observation were decreased up to 87% for mice receiving the OGFr-3 clonal cells and up to 78% for mice receiving the OGFr-22 clonal line, relative to tumour size for mice receiving wild-type or empty vector SKOV-3 cells.

Daily injections of OGF to groups of mice inoculated subcutaneously with genetically modified ovarian cancer tumour cells over-expressing OGFr repressed tumour growth substantially (Figure 2).[65] Tumour volumes were decreased in size up to 99% in clonal lines in comparison with those in mice receiving wild-type cells. Using orthotopic injections whereby cells were injected intraperitoneally to establish a more 'realistic' model of human ovarian cancer, there were up to 95% reductions in the number of tumour nodules in the intestines, liver and stomach compared with those observed in animals receiving empty vector cancer cells after 40 days. This suggests that human ovarian tumour cells with increased numbers of OGFr respond not only to endogenous OGF to suppress initial formation but are responsive to treatment with exogenous OGF. These data imply that OGF receptors may be the limiting factor regulating cancer progression. Examination of the tumours for late-stage apoptosis, DNA synthesis and blood vessel density showed that over-expression of OGFr (up to 146%) in tumour tissue had no effect on apoptosis, reduced DNA synthesis by two-thirds the levels in wild-type tumours and reduced new blood vessel formation (i.e. angiogenesis) in the tumours by as much as 86% relative to mice with wild-type human ovarian tumours.

To study the effects of tumour progression if OGFr is downregulated, human ovarian cancer cell lines that were transfected with OGFr shRNA, selectively cloned using hygromycin-B and expanded, demonstrated reductions in OGFr by as much as 73% relative to wild-type cells.[66] OGFr binding capacity was decreased in the clonal cell lines under-expressing OGFr, and expectedly, cell proliferation was accelerated. Clonal cell lines under-expressing OGFr had doubling times of ~25 h in comparison with wild-type cells with ~39 h doubling times. Treatment of ovarian cancer cells under-expressing OGFr with exogenous OGF did not inhibit their proliferation, nor decrease DNA synthesis, as the loss of receptor prevented the activity of OGF.

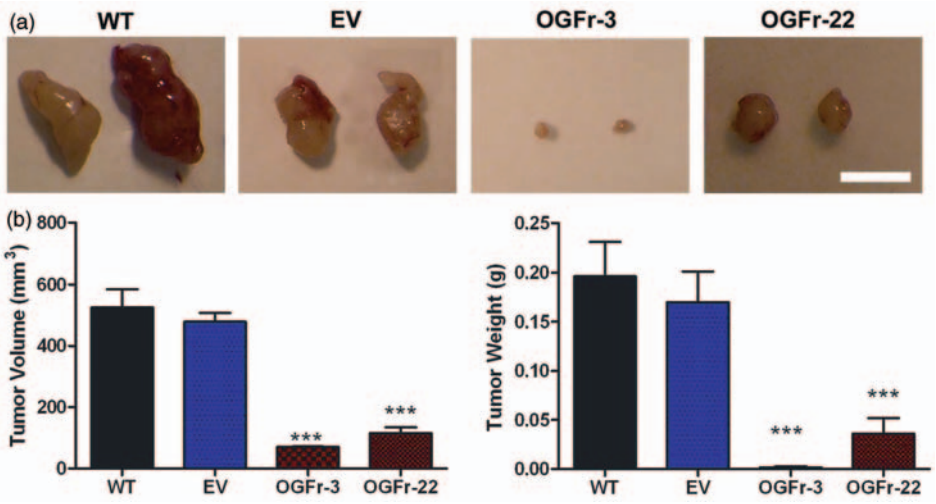

Figure 2 Representative images (a) of SKOV-3 tumours grown in female nude mice inoculated with human ovarian cancer cells stably transfected with clonal cell lines over-expressing OGFr (OGFr-3 and OGFr-22) or empty vector (EV); some mice were inoculated with wild-type (WT) SKOV-3 ovarian cancer cells. Tumours were removed 32 days after subcutaneous inoculation. Bar = 1 cm. (b) Terminal tumour volumes and tumour weights from mice developing tumours. Values represent means ± SE. Significantly different from WT and/or EV groups at ***$P < 0.001$. Adapted from Figure 5 in Ref. 65. (A color version of this figure is available in the online journal)

When clonal ovarian cancer cell lines under-expressing OGFr were inoculated into nude mice, tumour incidence was increased, latency times for tumour appearance were accelerated and tumour growth was enhanced relative to values from mice injected with wild-type or empty vector transfected ovarian cancer cells. Furthermore, tumours lacking OGFr were unresponsive to daily OGF injections. These data reveal that OGFr is a critical mediator of tumour progression and that the loss of the receptor accelerates cell proliferation *in vitro* and tumour growth *in vivo*.

In summary, the cell and molecular biology studies in culture and in animal models support that the OGF-OGFr axis is a tonically active biological pathway. The continuously active loop between native peptide and receptor is substantiated by the molecular manipulations of receptor and reinforces evidence from studies with neutralization of peptide by excess OGF antibody and transient knockdown of OGFr using siRNA. Diminishment of OGFr disengaged OGF-OGFr interfacing and rendered cells unresponsive to exogenous OGF.

OGF and OGFr in human patient samples of ovarian cancer

Preclinical studies suggest that human ovarian cancer may be managed by the OGF-OGFr axis, but studies with human tissues were necessary to confirm the presence and quantity of OGF and OGFr. In collaboration with oncologic gynaecologists, surgical specimens representative of malignant ovarian cancer and benign ovarian cysts were collected and assessed for levels of OGF and OGFr using semi-quantitative immunohistochemistry.[67] Normal human ovarian surface epithelial cells were collected from postmenopausal women undergoing hysterectomy and oophorectomy and used as comparative 'normals'. OGF and OGFr were identified in all tissues, but the amount of OGF present in cysts and malignant tumours, as measured immunohistochemically, was reduced 29% and 58%, respectively, from normal epithelial cells. Levels of OGFr were decreased 34% and 48% in cysts and malignant tumours, respectively, from normal levels measured in epithelial cell scrapings. Binding assays on cysts and tumour tissues indicated that malignant tumours had 5.4-fold fewer OGFr receptors than cysts; the small sample size of epithelial tissues prevented this pharmacological assay from being conducted. In summary, these studies document that OGFr receptor number is reduced in malignant ovarian tumour tissue relative to 'normal' epithelial cells, further supporting the hypotheses that the receptor is a requisite factor in regulating growth of ovarian cancer.

Opioid antagonists and blockade of the OGF-OGFr axis

The primary focus of preclinical studies on the role of the OGF-OGFr axis in the biology and treatment of cancer has been the inhibitory effectiveness of exogenous OGF. Studies on neuroblastoma,[18,27,45] pancreatic,[26,59] colon,[25] renal,[29] squamous cell carcinoma of the head and neck,[60–62] hepatocellular adenoma,[31] triple negative breast,[32] anaplastic thyroid[30] and ovarian[58,63,67] cancers have documented the presence of both peptide and receptor and have shown the requirement for intact OGFr protein to effectively inhibit growth by OGF.[33] However, it should be recognized that the OGF-OGFr axis obeys pharmacological principles of opioid–opioid receptor interactions, namely that opioid antagonists block the endogenous ligand–receptor interfacing. Studies using naltrexone to block the receptor associated with growth began more than three decades ago when the OGFr was not even isolated and identified.[38–42] Research showed that although naltrexone had no biological action of its own, the duration of time that the receptor is blocked yielded two totally different results.[39,44] In both mouse and rat animal studies, low dosages of the antagonist administered once daily resulted in suppressed cell proliferation in brain and body[44] and decreased growth

of cells in culture[45] and tumours in mice.[39,68] Conversely, higher dosages of naltrexone that blocked the receptor for the entire 24-h period resulted in enhanced growth. This conundrum was resolved when it was determined that opioid receptor blockade upregulated enkephalin peptide availability (secretion and/or production).[68] The increased levels of circulating enkephalins bound to OGFr and inhibited DNA synthesis and cell proliferation. The hypothesis that it was the duration of blockade, and not dosage of naltrexone, was confirmed by administration of low dosages of naltrexone multiple times daily to invoke a continuous blockade of opioid receptor and increased growth. Thus, low dosages or short-term exposure to naltrexone inhibited growth, and high dosages of naltrexone or continuous blockade accelerated growth. These concepts were applied to the tissue culture model for the first time using ovarian cancer cells.[69] Titration of naltrexone dosage to invoke a short-term receptor blockade *in vitro* was accomplished by incubation of cells with 10^{-5} M naltrexone for only 6 h and then changing media to remove residual naltrexone; this was termed 'short-term naltrexone'. Continuous exposure to naltrexone, and continuous blockade of OGFr, was accomplished by the addition of naltrexone to the replenished media (termed continuous naltrexone). The questions addressed by these studies were whether cancer treatment with low dosages of naltrexone could be envisioned as a feasible alternative to intravenous infusions of OGF that are currently required clinically. Exposure of SKOV-3 cultures to 10^{-5} M naltrexone, a concentration that was not toxic, for only 6 h, or for 6 h every other day, resulted in inhibited growth relative to control cultures. The inhibitory effects of a single 6 h exposure to naltrexone were observed for five days. Exposure to naltrexone daily for 1, 2 or 3 h decreased ovarian cancer cell number after three days by as much as 33% relative to controls. The specificity of naltrexone was tested by adding the short-acting opioid antagonist naloxone; naloxone exposure for 6 h or less also inhibited ovarian cancer cell number at 72 h. Comprehensive analyses of short-term naltrexone exposure and interactions with the OGF-OGFr axis revealed that short-term naltrexone obeyed the principles associated with OGF treatment including (i) altering DNA synthesis but not increasing apoptosis or necrosis, (ii) upregulating p16 and/or p21 cyclin-dependent inhibitory kinase pathways to repress cell division and (iii) utilizing OGFr, but not other classical opioid receptors, for the inhibitory action. Short-term naltrexone exposure in ovarian cancer cultures was accompanied by 18–32% increases in OGF peptide within the cells themselves as quantitated from immunocytochemical preparations after 24 and 72 h. Measurements of OGF secreted into the media revealed up to 48% more OGF secreted in cultures having a short-term exposure to naltrexone relative to controls. Sequential treatment of SKOV-3 and OVCAR-3 cells with short-term naltrexone followed 6 h later by OGF depressed cell number relative to sterile water treated controls by 35% and 61%, respectively, represented an additional ~40% reduction in cell number in comparison to treatment with only one of the agents. These data demonstrate that the OGF-OGFr axis can be targeted to repress ovarian cancer growth by pharmacological agents such as naltrexone. The duration of opioid receptor blockade, not the dosage of the antagonist, determined the response on cell proliferation. Cell cultures briefly (6 h) subjected to naltrexone had repressed cell proliferation, whereas more drug or drug more often, yielded detrimental effects by increasing cancer cell proliferation. Thus, in the case of opioid antagonist therapy for cancer, more naltrexone is not better.

Animal models to examine the effects of short-term exposure to naltrexone in culture utilized a low dosage of naltrexone (i.e. 0.1 mg/kg).[70] Beginning at the time of tumour appearance when the ovarian cancer was established, low-dose naltrexone (LDN) therapy repressed tumour progression up to 48% relative to control-treated mice in a non-toxic manner by reducing DNA synthesis and limiting angiogenesis in the tumour tissue. Addition of cisplatin and taxol to the LDN therapeutic regimen resulted in an additive inhibitory effect on tumourigenesis yielding even smaller tumours, enhanced depression of DNA synthesis and angiogenesis (Figure 3). Tumour volumes in mice receiving combinational therapies for established cancer were reduced over the course of 36 days by as much as 60% relative to controls. Combination of LDN with cisplatin, but not taxol, revealed some protective mechanisms as the body weights of mice receiving LDN along with their standard of care were reduced less than those reported for mice receiving individual chemotherapies. Analyses of tumours revealed upregulated OGFr receptor suggesting that the common pathway utilized between OGF and LDN (or intermittent naltrexone treatment *in vitro*) is the OGF-OGFr axis. These data suggest that short-term naltrexone, which is available as an oral tablet (i.e. low-dose naltrexone, 3–4.5 mg), could be an effective adjuvant therapy for ovarian cancer patients following surgery or standard chemotherapy.

Global summary of studies on the OGF-OGFr axis as a target for treatment of human ovarian cancer

In summary, our research supports and extends earlier observations about the OGF-OGFr axis in other cancers and confirms that this biological pathway is a determinant of human cancer progression. The OGF-OGFr axis can be an effective therapeutic target in three ways including (i) treatment with exogenous OGF – alone or in combination with chemotherapy,[71–74] (ii) endogenous OGF by low dosages of opioid antagonists such as naltrexone[75] or (iii) genetic manipulation to over-express OGFr.[74,76–79] With regard to human ovarian cancer, OGF and OGFr are present on cultured human ovarian cancer cells, in ovarian cancer cells xenografted into nude mice and in surgical samples of normal and malignant ovarian tissues. However, as human ovarian tissues become malignant, OGFr number is reduced. Exogenous OGF inhibits cell proliferation in a receptor-mediated, tonically active, reversible and dose-related manner. As recorded with normal[80] and neoplastic[81,82] tissues, OGF represses DNA synthesis of ovarian cancer cells by upregulation of p16 and/or p21 cyclin-dependent inhibitory kinases; apoptosis or necrosis are

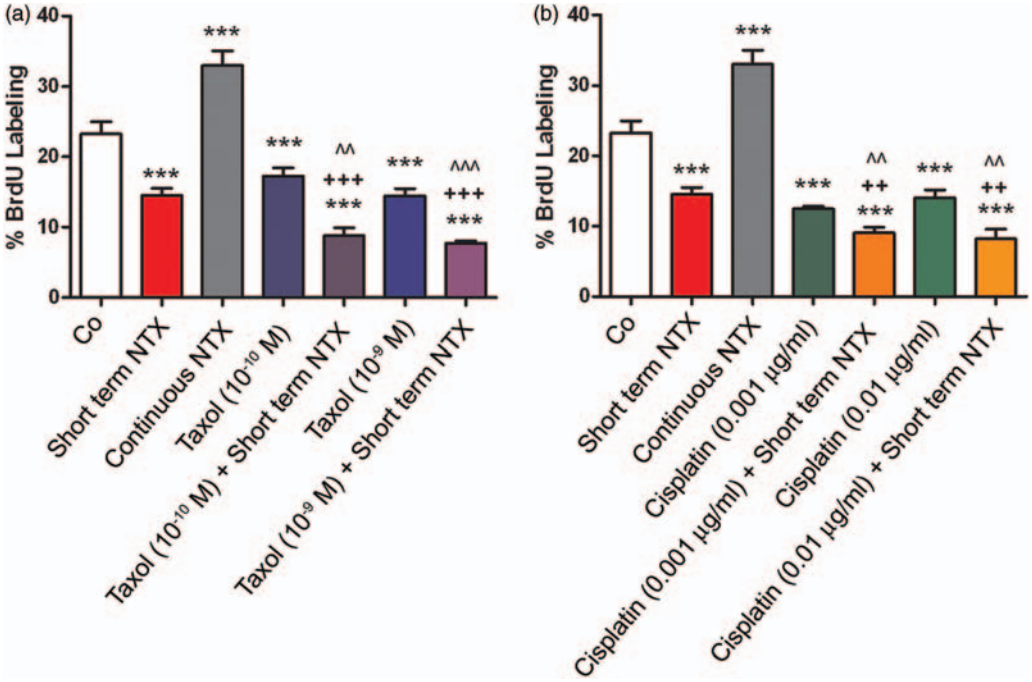

Figure 3 Enhanced growth inhibition by the combination of short-term naltrexone (NTX) treatment with taxol or cisplatin. DNA synthesis in SKOV-3 ovarian cancer cells treated for 120 h with short-term NTX (10^{-5} mol/L, 6 h every other day), continuous NTX (10^{-5} mol/L daily), taxol (10^{-9} or 10^{-10} mol/L), cisplatin (0.01 or 0.001 µg/mL), short-term NTX in combination with taxol or cisplatin, or sterile water (0.1 mL, Co). Media and compounds were replaced daily unless otherwise indicated. Values represent percentage of cancer cells labelled by BrdU (means ± SE). Significantly different from Co at $^{***}P < 0.001$, from short-term NTX treatment at $^{\wedge\wedge}P < 0.01$ and $^{\wedge\wedge\wedge}P < 0.001$, and from taxol or cisplatin treatment alone at $^{++}P < 0.01$ and $^{+++}P < 0.001$. Adapted from Figure 2 in Ref. 70. (A color version of this figure is available in the online journal)

not involved. Exogenous OGF inhibits tumour progression when treatment is initiated at the time of tumour appearance. Ovarian cancer cells manipulated to express more OGFr demonstrate repressed proliferation *in vitro* and *in vivo*. Tumour cells over-expressing OGFr and implanted into nude mice show delayed tumour appearance. Additional OGF treatment of wild-type cells or cells/tumours over-expressing OGFr show even more repression of growth suggesting that OGFr is the limiting agent in this biological axis. Cell lines with reduced OGFr multiply faster than wild-type ovarian cancer cells in culture, and when transplanted into nude mice. Exogenous OGF is also effective in combination with standard of care therapies such as taxol or cisplatin; combination therapy displays additive inhibitory effectiveness in culture and in the growth of tumours. OGF decreases angiogenesis in tumours and serves as a protectant against the loss of body weight by the nude mice treated with taxol or cisplatin. Short-term exposure to opioid antagonists such as naltrexone results in an elevation of both OGF and OGFr. When the antagonist is no longer available, growth inhibition occurs *in vitro* and *in vivo*. The short-term naltrexone treatment also could be combined with either taxol or cisplatin for additive inhibitory effect.

The research on the OGF-OGFr axis as a target for treatment of human ovarian cancer not only supported and extended many other studies documenting that exogenous OGF inhibited tumour cell replication but also provided new information on a novel therapy, LDN, for treatment of patients with cancer of the ovary. Moreover, the critical nature of the OGF-OGFr axis as a determinant of ovarian cancer progression has been substantiated in the preclinical setting for numerous cancers and now should be available for clinical use. While no clinical trials on OGF and ovarian cancer have been initiated, there have been two clinical trials on human pancreatic cancer (Phase I and Phase II)[83,84] and a Phase I trial on human hepatocellular carcinoma that recently closed. Dissemination of information on the efficacy and safety of OGF and LDN for cancer treatment hopefully will encourage controlled Phase II and Phase III clinical trials.

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REFERENCES

1. Romero I, Bast RC. Minireview: human ovarian cancer: biology, current management, and paths to personalizing therapy. *Endocrinology* 2012;**153**:1593–602

2. Leung PCK, Choi J-H. Endocrine signaling in ovarian surface epithelium and cancer. *Hum Reprod Update* 2007;**13**:143–62
3. Bast RC. CA125 and the detection of recurrent ovarian cancer: a reasonably accurate biomarker for a difficult disease. *Cancer* 2010;**116**:2850–3
4. Soslow RA. Histologic subtypes of ovarian carcinomas: an overview. *Int J Gynecol Pathol* 2008;**27**:161–74
5. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011;**61**:69–90
6. Howlander N, Noone AM, Krapcho M, Neyman N, Aminou R, Altekruse SF, Kosary CL, Ruhi J, Tatlovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA. (eds). SEER Cancer Statistics Review, 1975-2009, National Cancer Institute, Bethesda, MD. See http://seer.cancer.gov/statfacts.html.ovary.html.
7. American Cancer Society. Cancer Facts and Figures 2012. Altanta GA: American Cancer Society, 2012
8. Lynch HT, Watson P, Lynch J, Conway TA, Fili M. Hereditary ovarian cancer. Heterogeneity in age at onset. *Cancer* 1993;**71**(Suppl 2): 573–81
9. Yancik R. Ovarian cancer: age contrasts in incidence, histology, disease stage at diagnosis, and mortality. *Cancer* 1993;**71**(Suppl 2): 517–23
10. Penson RT, Shannon KE, Sharpless NE, Seiden MV. Ovarian cancer: an update on genetics and therapy. *Compr Ther* 1998;**24**:477–87
11. Piver MS, Goldberg JM, Tsukada Y, Mettlin CJ, Jishi MF, Natarajan N. Characteristics of familial ovarian cancer: a report of the first 1000 families in the Gilda Radner Familial Ovarian Cancer Registry. *Eur J Gynaecol Oncol* 1996;**17**:169–76
12. Wooster R, Newhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science* 1994;**265**:2088–90
13. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC, Tucker MA. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med* 1997;**336**:1401–8
14. See https://www.caring4cancer.com/go/ovarian/treatments
15. Zagon IS, McLaughlin PJ. Naltrexone modulates tumor response in mice with neuroblastoma. *Science* 1983;**221**:671–3
16. Zagon IS, McLaughlin PJ. Increased brain size and cellular content in infant rats treated with an opiate antagonist. *Science* 1983;**221**:1179–80
17. Zagon IS, McLaughlin PJ. Endogenous opioid systems regulate cell proliferation in the developing rat brain. *Brain Res* 1987;**412**:68–72
18. McLaughlin PJ, Zagon IS. Modulation of human neuroblastoma transplanted into nude mice by endogenous opioid systems. *Life Sci* 1987;**41**:1465–72
19. Zagon IS, Rhodes RE, McLaughlin PJ. Distribution of enkephalin immunoreactivity in germinative cells of developing rat cerebellum. *Science* 1985;**227**:1049–51
20. Zagon IS, Rhodes RE, McLaughlin PJ. Localization of enkephalin immunoreactivity in diverse tissues and cells of the developing and adult rat. *Cell Tiss Res* 1986;**246**:561–5
21. Meilandt WJ, Yu Gui-Qiu, Roberson ED, Palop JJ, Wu T, Scearce-Levie K, Mucke L. Enkephalin elevations contribute to neuronal and behavioral impairments in a transgenic mouse model of Alzheimer's disease. *J Neurosci* 2008;**28**:5007–17
22. McLaughlin PJ. Regulation of DNA synthesis of myocardial and epicardial cells in developing rat heart by [Met^5]-enkephalin. *Amer J Physiol* 1996;**271**:R122–9.
23. Zagon IS, Wu Y, McLaughlin PJ. The opioid growth factor, [Met^5]-enkephalin, and the ζ (zeta) opioid receptor are present in human and mouse skin and tonically act to inhibit DNA synthesis in the epidermis. *J Invest Dermatol* 1996;**106**:490–7.
24. Zagon IS, Sassani JW, Kane ER, McLaughlin PJ. Homeostasis of ocular surface epithelium in the rat is regulated by opioid growth factor. *Brain Res* 1997;**759**:92–102
25. Zagon IS, Hytrek SD, McLaughlin PJ. Opioid growth factor tonically inhibits human colon cancer cell proliferation in tissue culture. *Amer J Physiol* 1996;**271**:R511–8
26. Zagon IS, Smith JP, McLaughlin PJ. Human pancreatic cancer cell proliferation in tissue culture is tonically inhibited by opioid growth factor. *Int J Oncol* 1999;**14**:577–84
27. McLaughlin PJ, Zagon IS, Skitzki J. Human neuroblastoma cell growth in tissue culture is regulated by opioid growth factor. *Int J Oncol* 1999;**14**:373–80
28. McLaughlin PJ, Levin RJ, Zagon IS. Regulation of human head and neck squamous cell carcinoma growth in tissue culture by the opioid growth factor. *Int J Oncol* 1999;**14**:991–8
29. Bisignani GJ, McLaughlin PJ, Ordille SD, Jarowenko MJ, Zagon IS. Human renal cell proliferation in tissue culture is tonically inhibited by opioid growth factor. *J Urol* 1999;**162**:2186–91
30. McLaughlin PJ, Goldenberg D, Park SS, Conway A, Donahue RN, Zagon IS. Growth inhibition of thyroid follicular cell-derived cancers by the opioid growth factor (OGF) – opioid growth factor receptor (OGFr) axis. *BMC Cancer* 2009;**9**:369
31. Avella DM, Kimchi ET, Donahue RN, Tagaram HR, McLaughlin PJ, Zagon IS, Staveley-O'Carroll KF. The opioid growth factor-opioid growth factor receptor axis regulates cell proliferation of human hepatocellular cancer. *Amer J Physiol Regul Integr Comp Physiol* 2010;**298**:R459–66.
32. Zagon IS, Porterfield NK, McLaughlin PJ. Opioid growth factor (OGF) – opioid growth factor receptor (OGFr) axis inhibits proliferation of triple negative breast cancer. *Exp Biol Med* 2013, in press
33. Zagon IS, Donahue RN, McLaughlin PJ. Opioid growth factor-opioid growth factor receptor axis is a physiological determinant of cell proliferation in diverse human cancers. *Amer J Physiol* 2009;**297**:R1154–61
34. Zagon IS, McLaughlin PJ. An opioid growth factor regulates the replication of microorganisms. *Life Sci* 1992;**50**:1179–87
35. Zagon IS, McLaughlin PJ. Identification of opioid peptides regulating proliferation of neurons and glia in the developing nervous system. *Brain Res* 1991;**542**:318–23
36. Zagon IS, Hurst WJ, McLaughlin PJ. Identification of [Met^5]-enkephalin in developing, adult, and renewing tissues by reversed-phase high-performance liquid chromatography and radioimmunoassay. *Life Sci* 197;**61**:363–70
37. Mosnaim AD, Puente J, Wolf ME, Callaghan OH, Busch R, Diamond S. Studies of the in vitro human plasma degradation of methionine-enkephalin. *Gen Pharmacol* 1988;**19**:729–3
38. Zagon IS, McLaughlin PJ. Naltrexone modulates body and brain development in rats: a role for endogenous opioids in growth. *Life Sci* 1984;**35**:2057–64
39. Zagon IS, McLaughlin PJ. Duration of opiate receptor blockade determines tumorigenic response in mice with neuroblastoma: a role for endogenous opioid systems in cancer. *Life Sci* 1984;**35**:409–16
40. Zagon IS, McLaughlin PJ. Opiate-antagonist induced regulation of organ development. *Physiol Behav* 1985;**34**:507–11
41. Zagon IS, McLaughlin PJ. Opioid antagonist (naltrexone) modulation of cerebellar development: histological and morphometric studies. *J Neurosci* 1986;**6**:1424–32
42. Zagon IS, McLaughlin PJ. Modulation of murine neuroblastoma in nude mice by opioid antagonists. *J Natl Cancer Inst* 1987;**78**:141–7
43. Zagon IS, McLaughlin PJ. Opioid antagonist modulation of murine neuroblastoma: a profile of cell proliferation and opioid peptides and receptors. *Brain Res* 1989;**480**:16–28
44. Zagon IS, McLaughlin PJ. Naloxone modulates body and organ growth of rats: dependency on the duration of opioid receptor blockade and stereospecificity. *Pharmacol Biochem Behav* 1989;**33**:325–8.
45. Zagon IS, McLaughlin PJ. Opioid antagonist (naltrexone) stimulation of cell proliferation in human and animal neuroblastoma and human fibrosarcoma cells in culture. *Neuroscience* 1990;**37**:223–6
46. Zagon IS, Goodman SR, McLaughlin PJ. Characterization of opioid binding sites in murine neuroblastoma. *Brain Res* 1988;**449**:80–8
47. Zagon IS, Goodman SR, McLaughlin PJ. Characterization of zeta (ζ): a new opioid receptor involved in growth. *Brain Res* 1989;**482**:297–305
48. Zagon IS, Goodman SR, McLaughlin PJ. Demonstration and characterization of zeta (ζ), a growth-related opioid receptor, in a neuroblastoma cell line. *Brain Res* 1990;**511**:181–8

49. Zagon IS, Gibo DM, McLaughlin PJ. Zeta (ζ), a growth-related opioid receptor in the developing rat cerebellum: identification and characterization. *Brain Res* 1991;**551**:28–35
50. Zagon IS, Goodman SR, McLaughlin PJ. Zeta (ζ), the opioid growth factor receptor: identification and characterization of binding subunits. *Brain Res* 1993;**605**:50–6
51. Zagon IS, Verderame MF, Allen S, McLaughlin PJ. Cloning, sequencing, expression, and function of a cDNA encoding a receptor for the opioid growth factor, [Met^5]-enkephalin. *Brain Res* 1999;**849**:147–54
52. Zagon IS, Verderame MF, Allen SS, McLaughlin PJ. Cloning, sequencing, chromosomal location, and function of cDNAs encoding an opioid growth factor receptor (OGFr) in humans. *Brain Res* 2000;**856**:75–83
53. Zagon IS, Verderame MF, Zimmer WE, McLaughlin PJ. Molecular characterization and distribution of the opioid growth factor receptor (OGFr) in mouse. *Mol Brain Res* 2000;**84**:106–14
54. Zagon IS, Verderame MF, McLaughlin PJ. The biology of the opioid growth factor receptor (OGFr). *Brain Res Rev* 2002;**38**:351–76
55. Zagon IS, Ruth TB, Leure-duPree AE, Sassani JW, McLaughlin PJ. Immunoelectron microscopic localization of the opioid growth factor receptor (OGFr) and OGF in the cornea. *Brain Res* 2003;**967**:37–7
56. Zagon IS, Ruth TB, McLaughlin PJ. Nucleocytoplasmic distribution of opioid growth factor (OGF) and its receptor (OGFr) in tongue epithelium. *Anat Rec A: Discov Mol Cell Evol Biol* 2005;**282**:24–37
57. Cheng F, McLaughlin PJ, M F. Verderame, Zagon IS. Dependence on nuclear localization signals of the opioid growth factor receptor in the regulation of cell proliferation. *Exp Biol Med* 2009;**234**:532–41
58. Tanner MM, Grenman S, Koul A, *et al*. Frequent amplification of chromosomal region 20q12-q13 in ovarian cancer. *Clin Cancer Res* 2000;**6**:1833–9
59. Donahue RN, McLaughlin PJ, Zagon IS. Cell proliferation of human ovarian cancer is regulated by the opioid growth factor-opioid growth factor receptor axis. *Am J Physiol* 2009;**296**:R1716–25
60. McLaughlin PJ, Levin RJ, Zagon IS. Opioid growth factor (OGF) inhibits the progression of human squamous cell carcinoma of the head and neck transplanted into nude mice. *Cancer Lett* 2003;**199**:209–17
61. McLaughlin PJ, Stack BC, Braine KM, Ruda JD, Zagon IS. Opioid growth factor (OGF) inhibition of a human squamous cell carcinoma of the head and neck in nude mice: dependency on the route of administration. *Int J Oncol* 2004;**24**:227–32
62. McLaughlin PJ, Stack BC Jr, Levin RJ, Fedok F, Zagon IS. Defects in the OGF receptor (OGFr) in human squamous cell carcinoma of the head and neck. *Cancer* 2003;**97**:1701–10
63. Donahue RN, Zagon IS, McLaughlin PJ. The opioid growth factor inhibits established ovarian cancer in nude mice and can be combined with taxol or cisplatin to enhance growth inhibition. *J Cancer Ther* 2011;**2**:110–24
64. Donahue RN, McLaughlin PJ, Zagon IS. The opioid growth factor (OGF) and low dose naltrexone (LDN) suppress human ovarian cancer progression in mice. *Gynecol Oncol* 2011;**122**:382–8
65. Donahue RN, McLaughlin PJ, Zagon IS. Overexpression of the opioid growth factor receptor (OGFr) in human ovarian cancer cells downregulates cell proliferation *in vitro* and inhibits tumorigenesis in mice. *J Cancer Ther* 2011;**2**:579–94
66. Donahue RN, McLaughlin PJ, Zagon IS. Under-expression of the opioid growth factor receptor promotes progression of human ovarian cancer. *Exp Biol Med* 2012;**237**:167–77
67. Fanning J, Hossler CA, Kesterson JP, Donahue RN, McLaughlin PJ, Zagon IS. Expression of the opioid growth factor – opioid growth factor receptor axis in human ovarian cancer. *Gynecol Oncol* 2012;**124**:319–24
68. Zagon IS, McLaughlin PJ. 1995. Gene-peptide relationships in the developing rat brain: The response of preproenkephalin mRNA and [Met5]-enkephalin to acute opioid antagonist (naltrexone) exposure. *Mol Brain Res* 1995;**33**:111–20
69. Donahue RN, McLaughlin PJ, Zagon IS. Low-dose naltrexone targets the opioid growth factor-opioid growth factor receptor pathway to inhibit cell proliferation: mechanistic evidence from a tissue culture model. *Exp Biol Med* 2011;**236**:1036–50
70. Donahue RN, McLaughlin PJ, Zagon IS. Low dose naltrexone suppresses ovarian cancer and exhibits enhanced inhibition in combination with cisplatin. *Exp Biol Med* 2011;**236**:883–95
71. McLaughlin PJ, Jaglowski JR, Verderame MF, Stack BC, Leure-duPree AE, Zagon IS. Enhanced growth inhibition of squamous cell carcinoma of the head and neck by combination therapy of paclitaxel and opioid growth factor. *Int J Oncol* 2005;**26**:809–16
72. Jaglowski JR, Zagon IS, Stack BC, Verderame MF, Leure-duPree AE, Manning JD, McLaughlin PJ. Opioid growth factor enhances tumour growth inhibition and increases the survival of paclitaxel-treated mice with squamous cell carcinoma of the head and neck. *Cancer Chemother Pharmacol* 2005;**56**:97–104
73. Zagon IS, Jaglowski JR, Verderame MF, Leure-duPree AE, Smith JP, McLaughlin PJ. Combination chemotherapy with gemcitabine and biotherapy with opioid growth factor (OGF) enhances the growth inhibition of pancreatic adenocarcinoma. *Cancer Chemother Pharmacol* 2005;**56**:510–20
74. McLaughlin PJ, Stucki JK, Zagon IS. Modulation of the opioid growth factor ([Met^5]-enkephalin) – opioid growth factor receptor axis: novel therapies for squamous cell carcinoma of the head and neck. *Head Neck* 2012;**34**:513–19
75. Hytrek SD, McLaughlin PJ, Lang CM, Zagon IS. Inhibition of human colon cancer by intermittent opioid receptor blockade with naltrexone. *Cancer Lett* 1996;**101**:159–64
76. McLaughlin PJ, Zagon IS. Progression of squamous cell carcinoma of the head and neck is associated with down-regulation of the opioid growth factor receptor (OGFr). *Int J Oncol* 2006;**28**:1577–83
77. McLaughlin PJ, Verderame MF, Hankins JL, Zagon IS. Overexpression of the opioid growth factor receptor downregulates cell proliferation of human squamous carcinoma cells of the head and neck. *Int J Mol Med* 2007;**19**:421–8
78. Zagon IS, Verderame MF, Hankins JL, McLaughlin PJ. Overexpression of the opioid growth factor receptor potentiates growth inhibition in human pancreatic cancer cells. *Int J Oncol* 2007;**30**:775–83
79. McLaughlin PJ, Kreiner S, Morgan CR, Zagon IS. Prevention and delay in progression of human squamous cell carcinoma of the head and neck in nude mice by stable overexpression of the opioid growth factor receptor. *Int J Oncol* 2008;**33**:751–7
80. Cheng F, McLaughlin PJ, Verderame MF, Zagon IS. The OGF-OGFr axis utilizes the p16^{INK4a} and p21$^{WAF1/CIP1}$ pathways to restrict normal cell proliferation. *Mol Biol Cell* 2009;**20**:319–27
81. Cheng F, Zagon IS, Verderame MF, McLaughlin PJ. The opioid growth factor (OGF)-OGF receptor axis uses the p16 pathway to inhibit head and neck cancer. Cancer Res 2007;**67**:10511–18
82. Cheng F, McLaughlin PJ, Verderame MF, Zagon IS. The OGF-OGFr axis utilizes the p21 pathway to restrict progression of human pancreatic cancer. *Mol Cancer* 2008;**7**:5–17
83. Smith JP, Conter RL, Bingaman SI, Harvey JA, Mauger DT, Ahmad M, Demers LM, Stanley WB, McLaughlin, Zagon IS. Treatment of advanced pancreatic cancer with opioid growth factor: Phase I. *Anti Cancer Drug* 2004;**15**:203–9
84. Smith JP, Bingaman SI, Mauger DT, Harvey HA, Demers LM, Zagon IS. Opioid growth factor (OGF) improves clinical benefit and survival in patients with advanced pancreatic cancer. *Open Access J Clinical Trials* 2010;**2**:37–48