

Low dose naltrexone and cancer therapy

Dr Leonard Weinstock

Low dose naltrexone (LDN) has been used by physicians since 1984 in the setting of advanced cancers including gastrointestinal tumors such as colon cancer and pancreatic cancer. The effect of LDN has been studied in laboratory animal models with several types of cancer.

Naltrexone has been a FDA-approved drug since 1984. Many medicines are used for different purposes by clinicians than what the FDA has given an "indication" for usage. Naltrexone is an opioid antagonist or an "anti-narcotic" medication. There is accumulating experience with this medication that at low doses it helps treat a variety of inflammatory, autoimmune, and painful disorders. The role of the immune system is thought to be important in the control of cancer.

LDN could be considered in cases where chemotherapy is partly successful, when spread of cancer is significant, or when one is looking for something to try to improve the control of cancer by more natural means. It is not meant to replace chemotherapy.

To learn more, read about the original and subsequent use LDN in cancer treatment, see the website www.lowdosenaltrexone.org. Another website www.LDNscience.org gives all of the research publications on LDN. Our website www.gidoctor.net has our own research and educational presentations.

If you are interested in treatment with LDN plus alpha-lipoic acid, please make an appointment with Leonard Weinstock or Trish Myers – 314-997-0554 x102.

Information in clinical studies and basic science that support the use of LDN in cancer are as follows.

1. Clinical studies

The mu opioid receptor: A new target for cancer therapy?

Singleton PA, Moss J, Karp DD, Atkins JT, Janku F. Cancer. 2015;121:2681-8.

Mu opioids are among the most widely used drugs for patients with cancer with both acute and chronic pain as well as in the perioperative period. Several retrospective studies have suggested that opioid use might promote tumor progression and as a result negatively impact survival in patients with advanced cancer; however, in the absence of appropriate prospective validation, any changes in recommendations for opioid use are not warranted. In this review, the authors present preclinical and clinical data that support their hypothesis that the mu opioid receptor is a potential target for cancer therapy because of its plausible role in tumor progression. The authors also propose the hypothesis that peripheral opioid antagonists such as methylnaltrexone,

which reverses the peripheral effects of mu opioids but maintains centrally mediated analgesia and is approved by the US Food and Drug Administration for the treatment of opioid-induced constipation, can be used to target the mu opioid receptor.

Metabolic treatment of cancer: intermediate results of a prospective case series.
Schwartz L, Buhler L, Icard P, Lincet H, Steyaert JM. *Anticancer Res.* 2014;34:973-80.

BACKGROUND: The combination of hydroxycitrate and lipoic acid has been demonstrated by several laboratories to be effective in reducing murine cancer growth. **PATIENTS AND METHODS:** All patients had failed standard chemotherapy and were offered only palliative care by their referring oncologist. Karnofsky status was between 50 and 80. Life expectancy was estimated to be between 2 and 6 months. Ten consecutive patients with chemoresistant advanced metastatic cancer were offered compassionate metabolic treatment. They were treated with a combination of lipoic acid at 600 mg i.v. (Thioctacid), hydroxycitrate at 500 mg t.i.d. (Solgar) and low-dose naltrexone at 5 mg (Revia) at bedtime. Primary sites were lung carcinoma (n=2), colonic carcinoma (n=2), ovarian carcinoma (n=1), esophageal carcinoma (n=1), uterine sarcoma (n=1), cholangiocarcinoma (n=1), parotid carcinoma (n=1) and unknown primary (n=1). The patients had been heavily pre-treated. One patient had received four lines of chemotherapy, four patients three lines, four patients two lines and one patient had received radiation therapy and chemotherapy. An eleventh patient with advanced prostate cancer resistant to hormonotherapy treated with hydroxycitrate, lipoic acid and anti-androgen is also reported. **RESULTS:** One patient was unable to receive i.v. lipoic acid and was switched to oral lipoic acid (Tiobec). Toxicity was limited to transient nausea and vomiting. Two patients died of progressive disease within two months. Two other patients had to be switched to conventional chemotherapy combined with metabolic treatment, one of when had a subsequent dramatic tumor response. Disease in the other patients was either stable or very slowly progressive. The patient with hormone-resistant prostate cancer had a dramatic fall in Prostate-Specific Antigen (90%), which is still decreasing. **CONCLUSION:** These very primary results suggest the lack of toxicity and the probable efficacy of metabolic treatment in chemoresistant advanced carcinoma. It is also probable that metabolic treatment enhances the efficacy of cytotoxic chemotherapy. These results are in line with published animal data. A randomized clinical trial is warranted.

Long-term remission of adenoid cystic tongue carcinoma with low dose naltrexone and vitamin D3--a case report.

Khan A. *Oral Health Dent Manag.* 2014;13:721-4.

Naltrexone (ReVia®) is a long-acting oral pure opiate antagonist which is approved for the treatment of alcohol addiction as a 50mg per day tablet. The mechanism of action is complete opiate blockade, which removes the pleasure sensation derived from drinking alcohol (created by endorphins). Low Dose Naltrexone ("LDN") in the range of 3-4.5 mg per day has been shown to have the opposite effect - brief opiate receptor blockade with resulting upregulation of endogenous opiate production. Through the work of Bihari and Zagon, it has been determined that the level of the endogenous opiate methionine-

enkephalin is increased by LDN. Met-enkephalin is involved in regulating cell proliferation and can inhibit cancer cell growth in multiple cell lines. Increased met-enkephalin levels created by LDN thus have the potential to inhibit cancer growth in humans. Phase II human trials of met-enkephalin, case reports published by Berkson and Rubin, and the clinical experience of Bihari confirmed the potential role of LDN in treating pancreatic and other cancers. However, large scale trials are lacking and are unlikely to be funded given the current non-proprietary status of naltrexone. A case report is presented of successful treatment of adenoid cystic carcinoma as further evidence of LDN's potential as a unique non-toxic cancer therapy.

Revisiting the ALA/N (alpha-lipoic acid/low-dose naltrexone) protocol for people with metastatic and nonmetastatic pancreatic cancer: a report of 3 new cases.

Berkson BM, Rubin DM, Berkson AJ. *Integr Cancer Ther.* 2009;8:416-22.

The authors, in a previous article, described the long-term survival of a man with pancreatic cancer and metastases to the liver, treated with intravenous alpha-lipoic acid and oral low-dose naltrexone (ALA/N) without any adverse effects. He is alive and well 78 months after initial presentation. Three additional pancreatic cancer case studies are presented in this article. At the time of this writing, the first patient, GB, is alive and well 39 months after presenting with adenocarcinoma of the pancreas with metastases to the liver. The second patient, JK, who presented to the clinic with the same diagnosis was treated with the ALA/N protocol and after 5 months of therapy, PET scan demonstrated no evidence of disease. The third patient, RC, in addition to his pancreatic cancer with liver and retroperitoneal metastases, has a history of B-cell lymphoma and prostate adenocarcinoma. After 4 months of the ALA/N protocol his PET scan demonstrated no signs of cancer. In this article, the authors discuss the poly activity of ALA: as an agent that reduces oxidative stress, its ability to stabilize NF(k)B, its ability to stimulate pro-oxidant apoptotic activity, and its discriminative ability to discourage the proliferation of malignant cells. In addition, the ability of lowdose naltrexone to modulate an endogenous immune response is discussed. This is the second article published on the ALA/N protocol and the authors believe the protocol warrants clinical trial.

Reversal of signs and symptoms of a B-cell lymphoma in a patient using only low-dose naltrexone.

Berkson BM, Rubin DM, Berkson AJ. *Integr Cancer Ther.* 2007;6:293-6.

Single case report of inducing remission of stage III follicular lymphoma using LDN with alpha lipoic acid in a patient who had refused standard chemotherapy.

The long-term survival of a patient with pancreatic cancer with metastases to the liver after treatment with the intravenous alpha-lipoic acid/low-dose naltrexone protocol.

Berkson BM, Rubin DM, Berkson AJ. *Integr Cancer Ther.* 2006;5:83-9.

The authors describe the long-term survival of a patient with pancreatic cancer without any toxic adverse effects. The treatment regimen includes the intravenous alpha-lipoic acid and low-dose naltrexone (ALA-N) protocol and a healthy lifestyle program. The patient was told by a reputable university oncology center in October 2002 that there was little hope for his survival. Today, January 2006, however, he is back at work, free from symptoms, and without appreciable progression of his malignancy. The integrative protocol described in this article may have the possibility of extending the life of a patient who would be customarily considered to be terminal. The authors believe that life scientists will one day develop a cure for metastatic pancreatic cancer, perhaps via gene therapy or another biological platform. But until such protocols come to market, the ALA-N protocol should be studied and considered, given its lack of toxicity at levels reported. Several other patients are on this treatment protocol and appear to be doing well at this time.

2. Basic science studies

Naltrexone at low doses upregulates a unique gene expression not seen with normal doses: Implications for its use in cancer therapy.

Liu WM, Scott KA, Dennis JL, Kaminska E, Levett AJ, Dalglish AG. *Int J Oncol.* 2016;49:793-802.

It has been reported that lower doses of the opioid antagonist naltrexone are able to reduce tumour growth by interfering with cell signalling as well as by modifying the immune system. We have evaluated the gene expression profile of a cancer cell line after treatment with low-dose naltrexone (LDN), and assessed the effect that adapting treatment schedules with LDN may have on enhancing efficacy. LDN had a selective impact on genes involved with cell cycle regulation and immune modulation. Similarly, the pro-apoptotic genes BAD and BIK1 were increased only after LDN. Continuous treatment with LDN had little effect on growth in different cell lines; however, altering the treatment schedule to include a phase of culture in the absence of drug following an initial round of LDN treatment, resulted in enhanced cell killing. Furthermore, cells pre-treated with LDN were more sensitive to the cytotoxic effects of a number of common chemotherapy agents. For example, priming HCT116 with LDN before treatment with oxaliplatin significantly increased cell killing to 49 ± 7.0 vs. $14\pm 2.4\%$ in cultures where priming was not used. Interestingly, priming with NTX before oxaliplatin resulted in just $32\pm 1.8\%$ cell killing. Our data support further the idea that LDN possesses anticancer activity, which can be improved by modifying the treatment schedule.

Methionine enkephalin (MENK) improves lymphocyte subpopulations in human peripheral blood of 50 cancer patients by inhibiting regulatory T cells (Tregs).

Wang Q, Gao X, Yuan Z, et al. *Hum Vaccin Immunother.* 2014;10:1836-40..

MENK, a penta-peptide is considered as being involved in the regulatory feedback loop between the immune and neuroendocrine systems, with marked modulation of various functions of human immune cells. The aim of the present work was to investigate change of lymphocyte subpopulations in peripheral blood of 50 cancer patients before and after treatment with MENK. Peripheral blood mononuclear cells (PBMCs) of

peripheral blood from 50 cancer patients were isolated by density gradient centrifugation using Ficoll-Paque solution and cultured with MENK. We measured proliferation of total nucleated cells, subpopulations of individual CD4+T cells, CD8+T cells, CD4+CD25+ regulatory T cells (Treg), natural killer cells (NK) before and after treatment with 10(-12)M MENK in cell culture by flow cytometry (FCM). Our results indicated that MENK showed a strong inhibiting effect on Treg cells while it stimulated marked proliferation of other lymphocyte subpopulations. All data obtained were of significance statistically. It was therefore concluded that MENK could work as a strong immune booster with great potential in restoring damaged human immune system and we could consider MENK as a drug to treat cancer patients, whose immune systems are damaged by chemotherapy or radiotherapy. Furthermore we could consider MENK as a chemotherapy additive, which would sustain immune system of cancer patients during the process of chemotherapy to get maximized efficacy with minimized side effect.

Immunotherapy of cancer via mediation of cytotoxic T lymphocytes by methionine enkephalin (MENK).

Li W, Chen W, Herberman RB, et al. Cancer Lett. 2014;344:212-22.

The aim of this study was to investigate the immunological mechanisms by which synthetic methionine enkephalin (MENK) exerts therapeutic effects on tumor growth. Our findings in vivo or in vitro show that MENK treatment either in vivo or in vitro could up-regulate the percentages of CD8+T cells, induce markers of activated T cells, increased cytotoxic activity against mouse S180 tumor cells and increase secretion of IFN γ . In addition, the adoptively transferred CD8+T cells, after either in vitro or in vivo treatment with MENK, result in significantly increased survival of S180 tumor-bearing mice and significant shrinkage in tumor growth. Opioid receptors are detected on normal CD8+T cells and exposure to MENK leads to increased expression of opioid receptors. Interaction between MENK and the opioid receptors on CD8+T cells appears to be essential for the activation of CTL, since the addition of naltrexone (NTX), an opioid receptor antagonist, significantly inhibits all of the effects of MENK. The evidence obtained indicates that the MENK-induced T cell signaling is associated with a significant up-regulation of Ca $^{2+}$ influx into the cytoplasm and the translocation of NFAT2 into nucleus, and these signaling effects are also inhibited by naltrexone.

Opioid growth factor - opioid growth factor receptor axis inhibits proliferation of triple negative breast cancer.

Zagon IS. Exp Biol Med 2013;238:589-99

Triple negative breast cancer (TNBC) represents approximately 15% of the newly diagnosed cancers worldwide and is characterized by tissue lacking in estrogen, progesterone and human epidermal growth factor receptors. TNBC disproportionately affects younger women and women of colour, and new treatments are needed. The opioid growth factor (OGF) - opioid growth factor receptor (OGFr) axis is a determinant of cell proliferation in neoplasia, and OGF is an endogenously produced pentapeptide that inhibits cell replication by interacting with OGFr and upregulating cyclin-dependent

inhibitory kinase pathways thus reducing DNA synthesis. In these studies we investigated the presence and function of the OGF-OGFr axis in two human TNBC cell lines, as well as in breast cancer cell lines containing hormonal receptors. TNBC cell lines MDA-MD-231 and BT-20, as well as human breast cancer cells SK-BR-3 and MCF-7, were examined for the presence of pentapeptide and receptors, as well as their response to OGF. Specificity of peptide and receptor was confirmed by antibody neutralization and molecular studies to knockdown classical receptor protein. The requirement for protein transcription and translation and RNA transcription were investigated. Growth of TNBC cells in the presence of OGF and standard of care chemotherapeutic agent paclitaxel was evaluated to determine both efficacy and protective effects against toxicity. OGF treatment inhibited TNBC cells in a dosage related, receptor mediated, and reversible manner. OGF was the specific endogenous opioid to inhibit cell proliferation, and this was mediated by p21 cyclin dependent inhibitory kinase pathways, and required protein and RNA synthesis. OGFr was the specific receptor involved; both peptide and receptor were detected in all four cell lines. OGF treatment inhibited growth of all cancer cell lines evaluated, and reduced cell death in cultures exposed to paclitaxel. The OGF-OGFr axis is present and functioning in TNBC cell lines, and provides a novel biological pathway as potential therapy.

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.
McLaughlin PJ. Head Neck 2012;34:513-9.

BACKGROUND: The opioid growth factor (OGF)-OGF receptor (OGFr) axis is a constitutively expressed biologic pathway regulating cell proliferation of squamous cell carcinoma of the head and neck (SCCHN). This study investigated modulation of the OGF-OGFr system by (1) exogenous OGF, (2) upregulation of OGFr using imiquimod, or (3) intermittent opioid receptor blockade with a low dose of naltrexone on progression of established SCCHN. **METHODS:** Nude mice with visible human SCCHN SCC-1 tumors received (1) OGF or low-dose naltrexone either 1, 3, or 7 times/week or (2) imiquimod 1 or 3 times/week. Tumor growth and DNA synthesis were monitored. **RESULTS:** OGF and low-dose naltrexone increased the latency from visible to measurable tumors up to 1.6-fold. OGF, low-dose naltrexone, and imiquimod treatment markedly reduced tumor volume and weight, and decreased DNA synthesis in tumors. **CONCLUSIONS:** Modulation of the native OGF-OGFr regulatory network in SCCHN represents a novel nontoxic and highly efficacious approach for treatment of SCCHN.

Low-dose naltrexone targets the opioid growth factor-opioid growth factor receptor pathway to inhibit cell proliferation: mechanistic evidence from a tissue culture model.

Donahue RN, McLaughlin PJ, Zagon IS. Exp Biol Med. 2011;236:1036-50.

Naltrexone (NTX) is an opioid antagonist that inhibits or accelerates cell proliferation in vivo when utilized in a low (LDN) or high (HDN) dose, respectively. The mechanism of opioid antagonist action on growth is not well understood. We established a tissue culture model of LDN and HDN using short-term and continuous opioid receptor

blockade, respectively, in human ovarian cancer cells, and found that the duration of opioid receptor blockade determines cell proliferative response. The alteration of growth by NTX also was detected in cells representative of pancreatic, colorectal and squamous cell carcinomas. The opioid growth factor (OGF; [Met(5)]-enkephalin) and its receptor (OGFr) were responsible for mediating the action of NTX on cell proliferation. NTX upregulated OGF and OGFr at the translational but not at the transcriptional level. The mechanism of inhibition by short-term NTX required p16 and/or p21 cyclin-dependent inhibitory kinases, but was not dependent on cell survival (necrosis, apoptosis). Sequential administration of short-term NTX and OGF had a greater inhibitory effect on cell proliferation than either agent alone. Given the parallels between short-term NTX in vitro and LDN in vivo, we now demonstrate at the molecular level that the OGF-OGFr axis is a common pathway that is essential for the regulation of cell proliferation by NTX.

Low-dose naltrexone suppresses ovarian cancer and exhibits enhanced inhibition in combination with cisplatin.

Donahue RN, McLaughlin PJ, Zagon IS. *Exp Biol Med.* 2011;236:883-95.

Ovarian cancer is the leading cause of death from gynecological malignancies. Although initial therapeutic modalities are successful, 65% of these women relapse with only palliative treatments available thereafter. Endogenous opioids repress the proliferation of human ovarian cancer cells in vitro, and do so in a receptor-mediated manner. The present study examined whether modulation of opioid systems by the opioid antagonist naltrexone (NTX), alone or in combination with standard of care therapies (taxol/paclitaxel, cisplatin), alters human ovarian cancer cell proliferation in tissue culture and tumor progression in mice. Administration of NTX for six hours every two days, but not continuously, reduced DNA synthesis and cell replication from vehicle-treated controls in tissue culture. Moreover, brief exposure to NTX in combination with taxol or cisplatin had an enhanced anticancer action. Mice with established ovarian tumors and treated with a low dosage of NTX (LDN), which invokes a short period of opioid receptor blockade, repressed tumor progression in a non-toxic fashion by reducing DNA synthesis and angiogenesis but not altering cell survival. The combination of LDN with cisplatin, but not taxol, resulted in an additive inhibitory effect on tumorigenesis with enhanced depression of DNA synthesis and angiogenesis. LDN combined with cisplatin alleviated the toxicity (e.g. weight loss) associated with cisplatin. LDN treatment upregulated the expression of the opioid growth factor (OGF, chemical term ([Met(5)]-enkephalin) and its receptor, OGFr. Previous tissue culture studies have reported that OGF is the only opioid peptide with antiproliferative activity on ovarian cancer cells, with OGF action mediated by OGFr. Thus, the common denominator of intermittent opioid receptor blockade by short-term NTX or LDN on ovarian cancer proliferation and tumorigenesis recorded herein appears to be related to the OGF-OGFr axis. These preclinical data may offer a non-toxic and efficacious pathway-related treatment that can benefit patients with ovarian cancer.

The opioid growth factor-opioid growth factor receptor axis regulates cell proliferation of human hepatocellular cancer.

Avella DM. *Am J Physiol Regul Integr Comp Physiol.* 2010;298:R459-66

Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide, with a mortality rate approximating its incidence. Understanding the biology of these tumors, as well as treatment modalities, has been challenging. The opioid growth factor (OGF; [Met(5)]-enkephalin) and the OGF receptor (OGFr) form an endogenous growth-regulating pathway in homeostasis and neoplasia. In this investigation, we examined the relationship of the OGF-OGFr axis in HCC and define its presence, function, and mechanism. Using SK-HEP-1, Hep G2, and Hep 3B human HCC cell lines, we found that OGF and OGFr were present and functional. Exogenous OGF was observed to have a dose-dependent, reversible, and receptor-mediated inhibitory action on cell proliferation. Endogenous OGF was found to be constitutively produced and tonically active on cell replicative activities, with neutralization of this peptide accelerating cell proliferation. Silencing of OGFr using siRNA stimulated cell replication, even when exogenous OGF was added to the cultures, documenting its importance in mediating OGF activity. The mechanism of OGF-OGFr action on cell number was related to inhibition of DNA synthesis and not to apoptotic or necrotic pathways. Both OGF and OGFr were detected in surgical specimens of HCC, and no quantitative differences were recorded in peptide or receptor between pathological and normal specimens. These data are the first to report that the OGF-OGFr system is a native biological regulator of cell proliferation in HCC. The findings may provide important insight in designing treatment strategies for this deadly disease.

Cell proliferation of human ovarian cancer is regulated by the opioid growth factor-opioid growth factor receptor axis.

Donahue RN. *J Physiol Regul Integr Comp Physiol.* 2009;296:R1716-25.

Ovarian cancer is the leading cause of death from gynecological malignancies. Understanding the biology of these tumors, as well as treatment modalities, has been challenging. The opioid growth factor (OGF; [Met(5)]-enkephalin) and the OGF receptor (OGFr) form an endogenous growth-regulating pathway in homeostasis and neoplasia. In this investigation, we examined the relationship of the OGF-OGFr axis to ovarian cancer, and defined its presence, function, and mechanisms. Using OVCAR-3 and SKOV-3 ovarian cancer cell lines, we found that OGF and OGFr were present and functional. Exogenous OGF was observed to have a dose-dependent, serum-independent, reversible, and receptor-mediated inhibitory action on cell proliferation that was dependent on RNA and protein synthesis. The repressive effect of OGF on cell proliferation also was observed in SW626, CAOV-3, and HEY ovarian cancer cell lines. Endogenous OGF was found to be constitutively produced and tonically active on cell replicative activities, with neutralization of this peptide accelerating cell proliferation. Silencing of OGFr using siRNA technology stimulated cell replication, documenting its integral role. The mechanism of OGF-OGFr action on DNA synthesis was related to the cyclin-dependent kinase inhibitory pathway because knockdown of p16 or p21 in OVCAR-3 cells, and p21 in SKOV-3 cells, eliminated OGF's inhibitory effect on growth.

These data are the first to report that the OGF-OGFr system is a native biological regulator of cell proliferation in human ovarian cancer. This information will be important in designing treatment strategies for this deadly disease.

Growth inhibition of thyroid follicular cell-derived cancers by the opioid growth factor (OGF) - opioid growth factor receptor (OGFr) axis.

McLaughlin PJ. BMC Cancer. 2009;9:369

BACKGROUND: Carcinoma of the thyroid gland is an uncommon cancer, but the most frequent malignancy of the endocrine system. Most thyroid cancers are derived from the follicular cell. Follicular carcinoma (FTC) is considered more malignant than papillary thyroid carcinoma (PTC), and anaplastic thyroid cancer (ATC) is one of the most lethal human cancers. Opioid Growth Factor (OGF; chemical term - [Met5]-enkephalin) and its receptor, OGFr, form an inhibitory axis regulating cell proliferation. Both the peptide and receptor have been detected in a wide variety of cancers, and OGF is currently used clinically as a biotherapy for some non-thyroid neoplasias. This study addressed the question of whether the OGF-OGFr axis is present and functional in human thyroid follicular cell - derived cancer. **METHODS:** Utilizing human ATC (KAT-18), PTC (KTC-1), and FTC (WRO 82-1) cell lines, immunohistochemistry was employed to ascertain the presence and location of OGF and OGFr. The growth characteristics in the presence of OGF or the opioid antagonist naltrexone (NTX), and the specificity of opioid peptides for proliferation of ATC, were established in KAT-18 cells. Dependence on peptide and receptor were investigated using neutralization studies with antibodies and siRNA experiments, respectively. The mechanism of peptide action on DNA synthesis and cell survival was ascertained. The ubiquity of the OGF-OGFr axis in thyroid follicular cell-derived cancer was assessed in KTC-1 (PTC) and WRO 82-1 (FTC) tumor cells. **RESULTS:** OGF and OGFr were present in KAT-18 cells. Concentrations of 10-6 M OGF inhibited cell replication up to 30%, whereas NTX increased cell growth up to 35% relative to cultures treated with sterile water. OGF treatment reduced cell number by as much as 38% in KAT-18 ATC in a dose-dependent and receptor-mediated manner. OGF antibodies neutralized the inhibitory effects of OGF, and siRNA knockdown of OGFr negated growth inhibition by OGF. Cell survival was not altered by OGF, but DNA synthesis as recorded by BrdU incorporation was depressed by 28% in OGF-treated cultures compared to those exposed to sterile water. The OGF-OGFr axis was detected and functional in PTC (KTC-1) and FTC (WRO 82-1) cell lines. **CONCLUSION:** These data suggest that OGF and OGFr are present in follicular-derived thyroid cancers, and that OGF serves in a tonically active inhibitory manner to maintain homeostasis of cell proliferation. These results may provide a biotherapeutic strategy in the treatment of these cancers.

Opioid growth factor (OGF) inhibits anchorage-independent growth in human cancer cells.

Zagon IS, McLaughlin PJ. Int J Oncol. 2004;24:1443-8.

Opioid growth factor (OGF) is a native endogenous opioid peptide ([Met5]-enkephalin) that interacts with the OGF receptor (OGFr), and serves as a tonically active negative

growth factor in neoplasia. To inquire whether OGF modulates anchorage-independent growth, HT-29 human colon cancer cells were grown in soft agar and subjected to this peptide. In contrast to controls, HT-29 cells exposed to OGF had 57% fewer colonies, and these colonies were reduced in area by 75%. The changes induced by OGF were abolished by concomitant treatment with naloxone, indicating a receptor-mediated mechanism for peptide activity. Continuous blockade of opioid-receptor interactions with the potent and long-acting opioid antagonist, naltrexone (NTX), revealed an increase of 81 and 49% in the number and area, respectively, of colonies compared to control levels. These data suggest that OGF is tonically active in neoplastic cells growing in soft agar medium. HT-29 cells studied under anchorage-independent conditions were not influenced in growth by a variety of natural and synthetic opioids, including those selective for micro, delta, and kappa opioid receptors. Similar effects on anchorage-independent growth by OGF and NTX observed for HT-29 cells were recorded in pancreatic adenocarcinoma cells (Mia PaCa-2, Panc-1) and squamous cell carcinoma of the head and neck (CAL-27). These results using anchorage-independent conditions are consistent with previous data showing that OGF can markedly influence tumor growth in xenografts, and suggest that clonogenic assays can be utilized as indicators of tumorigenicity when tumor transplantation experiments are restricted.

Inhibition of human colon cancer by intermittent opioid receptor blockade with naltrexone.

Hytek SD, McLaughlin PJ, Lang CM, Zagon IS. *Cancer Lett.* 1996;101:159-64.

Nude mice inoculated with human colon cancer (HT-29) and receiving 0.1 mg/kg naltrexone (NTX) beginning immediately after tumor cell injection exhibited a marked retardation in tumorigenicity. This dosage of NTX, which blocked opioid receptors for 6-8 h/day, resulted in a delay of 2.4-fold in tumor appearance compared to control subjects. At the time (10 days) when all control mice had tumors, 80% of the mice in the 0.1 mg/kg NTX group had no signs of neoplasia. Binding capacity, but not affinity, of [³H][Met⁵]-enkephalin was reduced 85% of control levels in tumor tissue from mice of the 0.1 NTX group. Plasma, but not tumor tissue levels of [Met⁵]-enkephalin were elevated (2.5-fold) in contrast to control values. These results suggest that daily intermittent opioid receptor blockade with NTX provokes the interaction of opioids and receptors in the interval following drug availability, with opioids serving to inhibit tumorigenicity of human colon cancer.

Growth inhibition of thyroid follicular cell-derived cancers by the opioid growth factor (OGF) - opioid growth factor receptor (OGFr) axis.

McLaughlin PJ. *BMC Cancer.* 2009;9:369

BACKGROUND: Carcinoma of the thyroid gland is an uncommon cancer, but the most frequent malignancy of the endocrine system. Most thyroid cancers are derived from the follicular cell. Follicular carcinoma (FTC) is considered more malignant than papillary thyroid carcinoma (PTC), and anaplastic thyroid cancer (ATC) is one of the most lethal human cancers. Opioid Growth Factor (OGF; chemical term - [Met⁵]-enkephalin) and its receptor, OGFr, form an inhibitory axis regulating cell proliferation. Both the peptide and

receptor have been detected in a wide variety of cancers, and OGF is currently used clinically as a biotherapy for some non-thyroid neoplasias. This study addressed the question of whether the OGF-OGFr axis is present and functional in human thyroid follicular cell - derived cancer. METHODS: Utilizing human ATC (KAT-18), PTC (KTC-1), and FTC (WRO 82-1) cell lines, immunohistochemistry was employed to ascertain the presence and location of OGF and OGFr. The growth characteristics in the presence of OGF or the opioid antagonist naltrexone (NTX), and the specificity of opioid peptides for proliferation of ATC, were established in KAT-18 cells. Dependence on peptide and receptor were investigated using neutralization studies with antibodies and siRNA experiments, respectively. The mechanism of peptide action on DNA synthesis and cell survival was ascertained. The ubiquity of the OGF-OGFr axis in thyroid follicular cell-derived cancer was assessed in KTC-1 (PTC) and WRO 82-1 (FTC) tumor cells. RESULTS: OGF and OGFr were present in KAT-18 cells. Concentrations of 10^{-6} M OGF inhibited cell replication up to 30%, whereas NTX increased cell growth up to 35% relative to cultures treated with sterile water. OGF treatment reduced cell number by as much as 38% in KAT-18 ATC in a dose-dependent and receptor-mediated manner. OGF antibodies neutralized the inhibitory effects of OGF, and siRNA knockdown of OGFr negated growth inhibition by OGF. Cell survival was not altered by OGF, but DNA synthesis as recorded by BrdU incorporation was depressed by 28% in OGF-treated cultures compared to those exposed to sterile water. The OGF-OGFr axis was detected and functional in PTC (KTC-1) and FTC (WRO 82-1) cell lines. CONCLUSION: These data suggest that OGF and OGFr are present in follicular-derived thyroid cancers, and that OGF serves in a tonically active inhibitory manner to maintain homeostasis of cell proliferation. These results may provide a biotherapeutic strategy in the treatment of these cancers.