

HUMAN RENAL CELL CANCER PROLIFERATION IN TISSUE CULTURE IS TONICALLY INHIBITED BY OPIOID GROWTH FACTOR

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ABSTRACT

Purpose: Peptide growth factors alter cellular events by binding to specific receptors. One group of peptides, the endogenous opioids, is important in the growth of normal and neoplastic tissue. [Met⁵]enkephalin, also termed opioid growth factor (OGF), is a tonically active inhibitory factor that interacts with the OGF receptor, OGF_r, formerly identified as Greek zeta (ζ) and appears to be autocrine produced by human cancer cells. This study examined the hypothesis that OGF directly inhibits proliferation of renal cell carcinoma in tissue culture.

Materials and Methods: Human renal cancer cells (Caki-2) were grown using routine tissue culture techniques. A variety of natural and synthetic opioids including OGF, opioid antagonists, and opioid antibodies were added to renal cancer cell cultures to determine role of these peptides in renal cell carcinoma. The experiments were repeated in serum-free media, and with 4 other human renal cancer cell lines: Caki-2, A498, SN12C, and ACHN. Immunocytochemistry was performed to examine the presence of OGF and its receptor.

Results: OGF was the most potent opioid peptide to influence human renal cell carcinoma. OGF depressed growth within 12 hours of treatment, with cell numbers subnormal by up to 48% of control levels. OGF action was receptor mediated, reversible, not cytotoxic, neutralized by antibodies to the peptide, and detected in the human renal cell carcinoma lines examined. OGF appeared to be autocrine produced and secreted, and was constitutively expressed. Both OGF and its receptor were detected in these cells.

Conclusion: OGF tonically inhibits renal cancer cell proliferation in tissue culture, and may play a role in the pathogenesis and management of human renal cell cancer.

KEY WORDS: renal cell cancer, [Met⁵]-enkephalin, opioid growth factor, OGF, tissue culture

Renal cell carcinoma is the most common malignancy of the kidney, and is the seventh leading cause of cancer in the United States, accounting for 3% of malignancies in men.¹ The number of new cases in the United States in 1999 is projected to be 30,000, with an estimated 11,900 deaths.¹ Worldwide, 150,000 new cases occur annually (1.9% of the world total), and 78,000 deaths are reported.² Approximately a third of the cases of renal cell cancer have metastatic disease at presentation and 90% or more of these patients die within five years of diagnosis.³ Hormonal and chemotherapeutic agents have yet to prove effective with respect to renal cell carcinoma.^{4–6} Modifiers of biological responses such as interferons and interleukins have been used as systemic treatment for advanced renal cell carcinoma but most patients do not respond and clear evidence of improved survival with this treatment is yet to be forthcoming.^{4–6} Complete surgical removal of the neoplasm is essential, and failure to eradicate the primary tumor will most likely lead to advancing disease and death. However, 50% of those resected are expected to have a relapse during the course of the disease.⁶ Despite advances in the area of molecular genetics, the use of immune based therapy for advanced disease, and comprehension of the limitations of traditional cytotoxic chemotherapy, efforts are needed to elucidate the etiology, pathogenesis, and management of renal cell carcinoma.⁵

Peptide growth factors are regulatory proteins that bind to

specific cell receptors and govern the response of the cell to injury and mediate the highly coordinated process of cell growth, differentiation and death.⁷ Peptide growth factors have been shown to alter cellular events in tumors and several peptide growth factors are known to play a role in urological malignancy.^{8–13} For example, transforming growth factor α and β , epidermal growth factor and heparin-binding growth factor are known peptide growth factors expressed in renal cell carcinoma. The role of these growth factors in modulating tumorigenicity of renal cell carcinoma needs to be clarified.

One group of peptides, the endogenous opioids, appears to be important in the growth of normal, neoplastic, renewing and healing tissues.^{14–19} The pentapeptide [Met⁵]enkephalin, has been identified as an endogenous opioid directly involved in growth processes, serving as a constitutive negative regulator in a wide variety of cells and tissues. Cell proliferation, as well as aspects of cell migration, differentiation and survival are influenced by this growth peptide. In view of the growth-related function of this opioid peptide, [Met⁵]enkephalin has been termed opioid growth factor (OGF). Unlike neuromodulation which involves classical opioid receptors (for example, μ , δ) OGF interfaces with the OGF receptor (OGF_r, formerly identified as Greek zeta) to modulate growth. Recent studies have shown that OGF inhibited human gastrointestinal cancer cell growth in tissue culture and also prevented the incidence and retarded the growth of human colon and pancreatic cancer cells in nude mice.^{16–19}

The present study explored the question of whether endogenous opioids function as modulators of growth in human

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renal cell carcinoma. Using a tissue culture model of Caki-2 cells, identification, characterization, and ubiquity of the opioid peptides involved with cell generation were determined. Moreover, the presence and location of the opioid peptide and its receptor associated with growth of human renal cell carcinoma were established.

MATERIALS AND METHODS

Cells and cell culture. The human renal carcinoma cell lines Caki-2, Caki-1, A498, and ACHN were obtained from the American Type Culture Collection (Manassas, VA), and the human renal carcinoma cell line SN12C was a gift from Dr. I. J. Fidler (The University of Texas, M.D. Anderson Cancer Center, Houston, TX). The Caki-2 cell line was derived from a primary clear cell renal carcinoma in a 69-year-old male.²⁰ The Caki-1 cell line was derived from a metastatic skin lesion containing renal adenocarcinoma in a 49-year-old male. The A498 cell line was derived from a 52-year-old female with kidney carcinoma, and the ACHN cell line was derived from a malignant pleural effusion in a 22-year-old male with metastatic renal adenocarcinoma. The SN12C cell line was derived from a primary renal tumor, diagnosed as granular cell-type renal cell carcinoma with extensive invasion of perinephric fat, subsequent to a radical nephrectomy in a 43-year-old male.²¹

Caki-2 cells were maintained in McCoy's 5A media (modified) with L-glutamine. The Caki-1, A498, and ACHN cells were incubated in RPMI 1640 media with L-glutamine and sodium pyruvate. SN12C cells were cultured in Eagle's media with L-glutamine, sodium pyruvate and nonessential amino acids. All media contained 10% fetal calf serum, unless otherwise noted, and the cells were grown in a humidified atmosphere of 5% CO₂/95% air at 37°C. In some experiments Caki-2 cell cultures were maintained in McCoy's 5A media without fetal calf serum.

Growth assays. Equivalent amounts of cells (approximately 20,000 cells/well) were seeded into 9.6 cm² wells (6-well plates, Falcon) and counted 24 hours later to determine plating efficiency. Compounds or sterile media (vehicle) were added beginning 24 hours after seeding (=0 hours) and both media and drugs were replaced daily. All drugs were prepared in sterile media and dilutions represent final concentrations of the compounds. At designated times, cells were harvested by trypsinization with 0.05% trypsin/0.53 mM EDTA, centrifuged and counted with a hemacytometer. Cell viability was determined by trypan blue staining. At least two aliquots per well were counted and two wells per treatment group at each time point were sampled.

Immunocytochemistry. To examine the presence of OGF, [Met⁵]-enkephalin, and the OGF receptor, log-phase Caki-2 cells were plated onto round coverglasses and examined 72 hours after seeding. Cells were fixed and permeabilized in 95% ethanol and acetone at -20°C, rinsed in Sorenson's phosphate buffer (SPB), and blocked with SPB and 3% normal goat serum and 0.1% Triton X-100 at room temperature for 15 minutes. Ammonium sulfate purified anti-[Met⁵]-enkephalin immunoglobulin G (IgG) or anti-OGFr IgG were diluted (1:100) in SPB with 1% normal goat serum in 0.1% Triton X-100 and added for 18 hours at 4°C. Coverglasses were washed and incubated with goat anti-rabbit IgG (1:100) conjugated to rhodamine and viewed with fluorescence microscopy. Antibodies to [Met⁵]-enkephalin (CO-172) and OGFr (AO-440) were generated in the laboratory and have been fully characterized.^{18,19} Some coverglasses containing cells were incubated with secondary antibody only, or antisera preabsorbed overnight with either an excess of [Met⁵]-enkephalin or OGFr binding subunits and secondary antibody, and served as controls.

Chemicals. The following compounds were obtained from the indicated sources: [Met⁵]-enkephalin, [Leu⁵]-enkephalin,

[D-Pen^{2,5}]-enkephalin (DPDPE), [D-Ala,²MePhe,⁴Glyol⁵]-enkephalin (DAMGO), β -endorphin, naltrexone (NTX), naloxone (NAL), dynorphin A1-8, BAM12P, [Met,⁵ Arg,⁶ Phe⁷]-enkephalin (heptapeptide), [Met,⁵ Arg,⁶ Gly,⁷ Leu⁸]-enkephalin (octapeptide, proenkephalin), Sigma (St. Louis, MO); (+)-SKF-10,047 (SKF-10,047), National Institute on Drug Abuse (Rockville, MD); U-69,593, Biomol (Plymouth Meeting, PA); ICI-174,864, Cambridge Research Biochemicals (Valley Stream, NY); ethylketocyclazocine (EKC), Sterling-Winthrop (Rensselaer, NY).

Statistics. Cell numbers were analyzed using analysis of variance with subsequent comparisons made with Newman-Keuls tests.

RESULTS

Opioid antagonists and Caki-2 growth. Experiments were performed to ascertain whether opioid peptides play a role in cell proliferation of human renal cancer cells. Using a paradigm of blocking opioid-receptor interaction with a long-acting opioid antagonist, addition of 10⁻⁶ M NTX to Caki-2 cultures resulted in a 84% increase in growth from control levels within 24 hours of instituting opioid receptor blockade (fig. 1). Cell number was increased 40% to 76% from control values 48 to 96 hours after initiation of drug exposure (fig. 1).

Determination of the opioid peptide(s) related to growth of renal cancer. To determine which opioid peptide(s) is/are related to growth, cultures of Caki-2 cells were treated with 10⁻⁶ M concentrations of a variety of opioid compounds (synthetic and natural). Many of these drugs were specific for opioid receptors, and included: DAMGO (μ), DPDPE and ICI-174,864 (δ), dynorphin A1-8, U-69-593, and EKC (κ), β -endorphin (ϵ), and SKF-10,047 (σ). Cells were counted after 48 hours with drug (both drug and media were replaced daily). The results showed that the native pentapeptide [Met⁵]-enkephalin had the most potent action on cell proliferation, decreasing growth to 57% of control values (table). Heptapeptide, octapeptide, and [Leu⁵]-enkephalin exhibited inhibitory properties on the replication of Caki-2 cells. All other compounds, used at concentrations of 10⁻⁶ M, had no influence on growth. As mentioned earlier, [Met⁵]-enkephalin has been previously termed the opioid growth factor (OGF) to designate its function as a growth factor in neural, non-neural, normal, and tumorigenic tissues and cells;¹⁴⁻¹⁹ such terminology was employed in this report.

Evaluation of OGF and the growth of Caki-2 cells. To

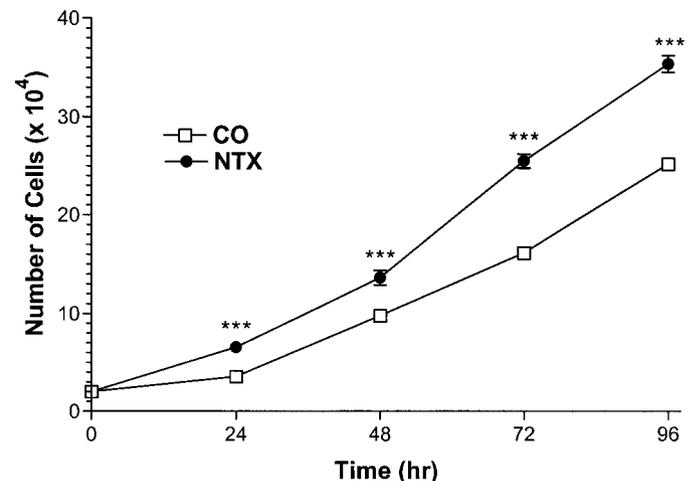


FIG. 1. Growth of Caki-2 cells subjected to 10⁻⁶ M naltrexone (NTX). Twenty-four hours after seeding (0 hours), NTX or an equivalent volume of vehicle (CO) was added; media and NTX were replaced daily. Data represent means \pm SE for at least two aliquots per well from three wells per group per time point. Significantly different from controls at $p < 0.001$ (***)

Opioid compounds that influence the growth of Caki-2 human renal cancer cells in culture

Compound	Structure	% Control
[Met ⁵]-enkephalin	Tyr-Gly-Gly-Phe-Met	57%***
[Leu ⁵]-enkephalin	Tyr-Gly-Gly-Phe-Leu	60%***
Heptapeptide	Tyr-Gly-Gly-Phe-Met-Arg-Phe	61%***
Octapeptide	Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu	80%***

*** Significantly different from controls at $p < 0.001$.

further define the extent of OGF action in Caki-2 human renal carcinoma cell growth, the effect of a variety of doses of OGF on the growth of log-phase cells was monitored at 48 and 72 hours after drug exposure (drug and media were replaced daily). At 48 hours cultures treated with 10^{-4} M to 10^{-7} M OGF were reduced from control levels by 27 to 44%; the group given 10^{-8} M and 10^{-9} M were subnormal by 11% and 5%, respectively, but these reductions were not significantly different from control values (data not shown). At 72 hours, all dosages of OGF utilized (10^{-4} M to 10^{-9} M) significantly reduced the number of Caki-2 cells from control values (fig. 2, A).

To investigate the magnitude and duration of the inhibitory effects of OGF on human renal cancer cell growth, log-

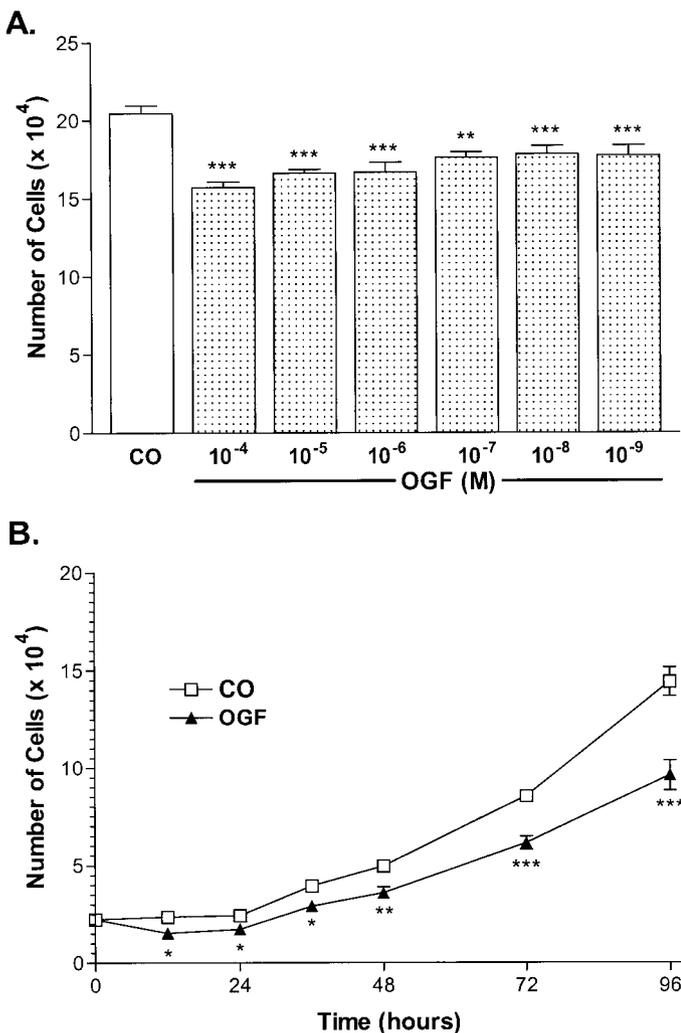


FIG. 2. A, growth of Caki-2 cells subjected to various concentrations of opioid growth factor (OGF) for 72 hours. B, growth of Caki-2 cells subjected to 10^{-6} M OGF or vehicle over 96-hour period of time. OGF or equivalent volume of vehicle (CO) was added 24 hours after seeding, and media and OGF were replaced daily. Data represent means \pm SE for at least two aliquots per well from three wells per group, and for each group/time point (B). Significantly different from controls at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

phase cells were exposed to 10^{-6} M OGF for 96 hours (drug and media replaced daily); these data are presented in fig. 2, B. Analysis of growth curves indicated that cell number was significantly depressed beginning 12 hours after addition of drug; cell number in the OGF wells was 35% less than control levels at 12 hours, and 26% to 33% at 24, 36, 48, 72, and 96 hours (fig. 2, B).

To study whether the inhibitory effect of OGF on cell number in Caki-2 human renal cancer cells was mediated by an opioid receptor, cultures were treated with both OGF (10^{-6} M) and the short acting opioid receptor antagonist NAL (10^{-6} M), only NAL (10^{-6} M), only OGF (10^{-6} M), or vehicle. Cells were counted 48 hours later; drugs and media were replaced daily. OGF inhibited cell number by 47% from control values, whereas concomitant administration of OGF and the antagonist blocked the inhibitory effects of OGF (fig. 3). Naloxone alone had no influence on cell growth.

Reversibility of the inhibitory growth effects of OGF. The inhibitory effect of OGF on cell number could be related to cytotoxicity, rather than a biological interaction of the peptide on mechanisms of cell proliferation. To address the question of whether the effects of OGF on cell number were permanent, a study was designed to determine if the inhibitory alterations of OGF could be reversed (fig. 4). Cultures of Caki-2 cells were exposed for 24 hours to 10^{-6} M OGF, and the number of OGF treated cells was reduced 47% from control levels (fig. 4). Twenty-four hours after the OGF media was removed and replaced with control media, the cultures contained 40% more cells than those continuing to receive OGF. Forty-eight and 72 hours after removal of OGF and replacement with control media, cell number was 29% and 36%, respectively, greater than the cultures maintained with OGF. Calculation of the increase in number of cells per hour during log-phase growth (between 24 and 48 hours after drug administration) revealed that the cells grew at the following rates: 514 cells/hour for the controls, 475 cells/hour for cells exposed to OGF for only 24 hours and removal of the drug, and 191 cells/hour for the cells continually exposed to OGF.

Antibody blockade of OGF inhibition. If OGF is an inhibitory peptide that is tonically active in Caki-2 cell cultures, then blockade of OGF-receptor interaction with an antibody to OGF would be predicted to increase cell number (fig. 5). Cultures of log-phase Caki-2 cells treated with 1:100 dilution of anti-OGF or non-immune rabbit IgG immunoglobulin (each culture received approximately 34 μ g. of antibody or

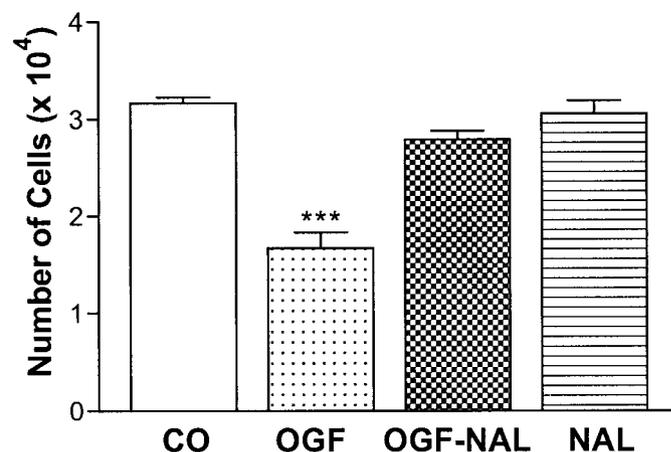


FIG. 3. Number of cells in Caki-2 cultures 48 hours after being subjected to 10^{-6} M concentrations of OGF, OGF and the opioid antagonist naloxone (OGF-NAL), NAL alone (NAL), or an equivalent volume of vehicle (CO). Compounds were added 24 hours after seeding, and both media and drugs were replaced daily. Data represent means \pm SE for at least two aliquots per well from three wells per group per time point. Significantly different from controls at $p < 0.001$ (***)

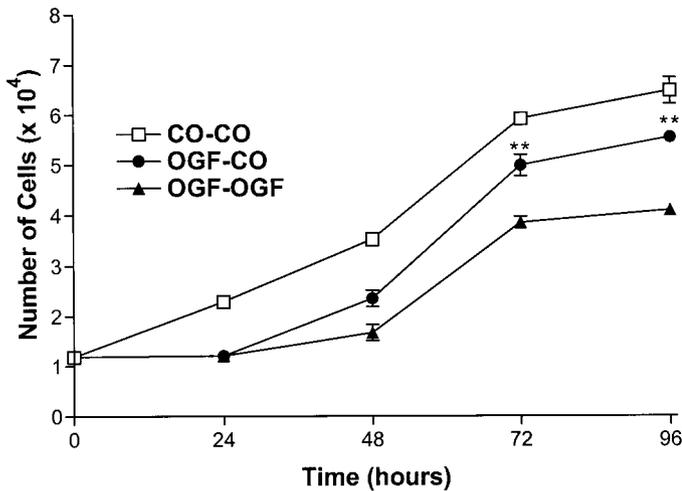


FIG. 4. Reversibility of OGF-induced growth inhibition in Caki-2 human renal cancer cells. Cells were seeded and 24 hours later (0 hours), OGF (10^{-6} M) or an equivalent volume of vehicle was added. Twenty-four hours later, some OGF-treated cultures continued to receive OGF (OGF-OGF), whereas other OGF-treated cultures were rinsed and incubated with media containing vehicle only (OGF-CO). Control cultures received vehicle daily (CO). Compounds and media were changed daily. Data represent means \pm SE for at least two aliquots per well from three wells per group per time point. OGF-CO group differed significantly from OGF-OGF group at $p < 0.01$ (***) at 72 and 96 hours after initial treatment with the compound. OGF-OGF group differed from CO group at $p < 0.01$ at every time point examined (no symbols indicated).

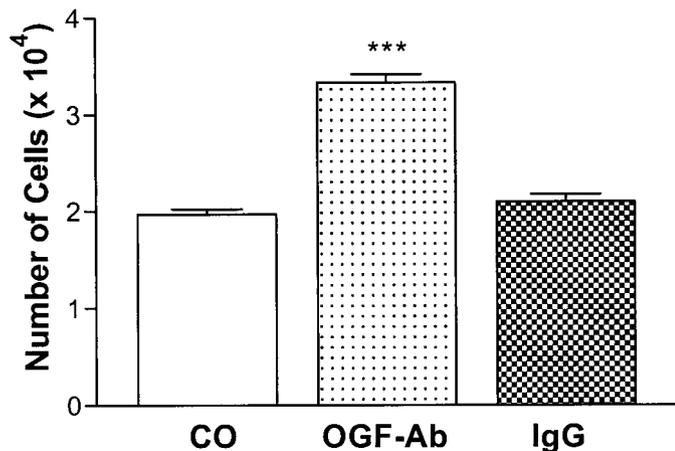


FIG. 5. Cultures of Caki-2 human renal cancer cells exposed to polyclonal antibody to OGF generated in rabbits (OGF-Ab), non-immune rabbit immunoglobulin (IgG), or vehicle (CO); cells were examined 48 hours later. Cells were seeded and allowed to grow for 24 hours before antibody was added. Media and compounds were replaced daily. Data represent means \pm SE for at least two aliquots per well from three wells per group. Significantly different from both CO and the non-immune IgG groups at $p < 0.001$ (***)

IgG immunoglobulin) were counted 48 hours later. The number of cells in cultures treated with anti-OGF IgG was 69% greater than in control cultures, but no differences in cell number were noted between non-immune IgG-treated and control cultures.

Opioid peptide modulation of Caki-2 human renal cancer cells in serum free media. The experiments showing that opioid peptides and, in particular, OGF influenced the growth of Caki-2 renal cancer cells were conducted in serum-containing media. To eliminate any confounding variables introduced by the serum, Caki-2 cells were adapted to grow in serum-free media and subjected to NTX or OGF. Adaptation to conditions of serum-free media took place over a four week period by gradually reducing serum from 10, 5, 2.5, 1.25

to 0%. Cells were plated in equal numbers and treated with 10^{-6} M concentrations of OGF, OGF and NAL, NAL, NTX, or an equivalent volume of vehicle. Cells were counted 72 hours later (fig. 6). OGF inhibited growth by 72% from control levels, and NTX increased cell number by 77%. OGF-NAL and NAL had no influence on cell growth.

Ubiquitous nature of the growth inhibitory effects of OGF on human renal cell carcinoma. To examine the ubiquity of the inhibitory effect of OGF on renal cell carcinoma, 4 other human renal cancer cell lines were investigated: Caki-1, A498, ACHN, and SN12C (fig. 7). Administration of 10^{-6} M OGF for 24 hours showed that OGF-treated cultures had 16% to 38% less cells than control cultures; these values were significantly different from control levels.

Immunolocalization of OGF and the OGF receptor in human renal carcinoma cells. Antibodies to OGF and to the OGF_r were used with immunocytochemistry to determine the presence and location of this growth-related peptide and its receptor in Caki-2 cells (fig. 8). Both antibodies provided a similar pattern of immunocytochemical labeling in the cells. The cytoplasm, but not the nuclei, of the Caki-2 cells was immunofluorescent. No staining was recorded in control specimens processed with antibody preabsorbed with respective antigens or in samples incubated with secondary antibody only (data not shown).

DISCUSSION

This study reports for the first time that endogenous opioid peptides are determinants of the growth of human renal cell carcinoma. Assessment of a wide variety of opioid-like compounds established that [Met⁵]-enkephalin (termed OGF), and to a lesser extent three related peptides, modulated the course of human renal cell carcinoma growth in tissue culture. A wide variety of synthetic and natural neuropeptides related to the μ , δ , κ , σ , and ϵ opioid receptors were not involved with functions related to cell generation. The data disclosed that OGF exerted a dose-dependent action, and influenced growth as measured by cell number within 24 hours of peptide exposure. The growth suppression recorded was mediated by opioid receptors because an opioid antagonist of short duration and low potency, naloxone, blocked the depression in cell acquisition but by itself did not have any activity on cell replication. Further experiments revealed that OGF was tonically active because persistent blockade of opioid-receptor interaction with the potent opioid antagonist, naltrexone, or the removal of OGF using antibodies to this peptide resulted in an increase in the number of cells. The

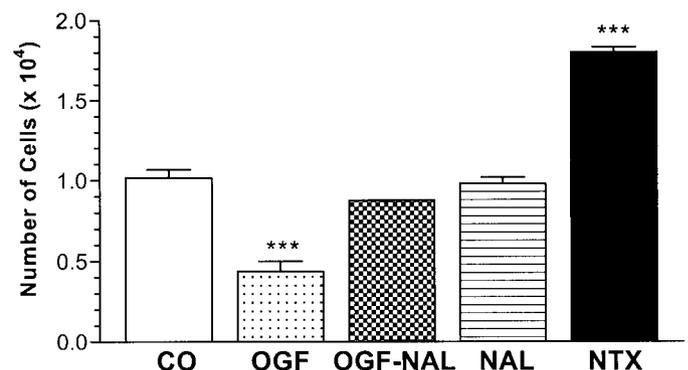


FIG. 6. Effects of OGF and NTX on number of Caki-2 human renal cancer cells grown in serum-free media after 48 hours. Twenty-four hours after seeding Caki-2 cells that had been acclimated to serum-free conditions, cultures were exposed to OGF, OGF-NAL, NAL alone, or NTX at a concentration of 10^{-6} M, or an equivalent volume of vehicle. Media and compounds were replaced daily. Data represent means \pm SE for at least two aliquots per well from two wells per group per time point. Significantly different from controls at $p < 0.001$ (***)

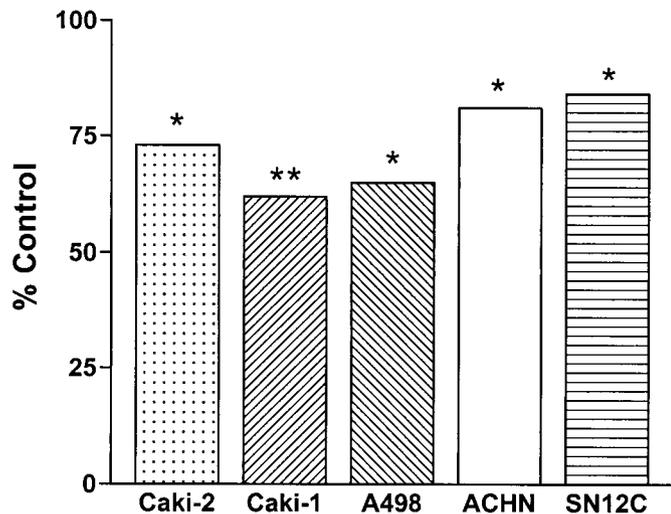


FIG. 7. Effects of OGF (10^{-6} M) on variety of human renal cancer cell lines. Compounds were added 24 hours after seeding, and both media and drug were replaced daily. Data represent percentage of control levels for at least two aliquots per well from three wells per group. Significantly different from control values at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***)

effects of OGF were neither detrimental nor cytotoxic to the human renal carcinoma cells but appeared to be cytostatic. Trypan blue staining of dead cells in OGF-treated cultures showed no changes in the number of cells collected from the culture vessels or in the media from control levels, suggesting that OGF exposure does not lead to cell death. The effects of OGF also were reversible when cells were transferred from OGF-containing media to fresh media without compound. Additionally, OGF activity was not dependent on the presence of serum, because opioid modulation of growth was detected under serum-free conditions. Finally, the regulatory role of OGF in growth was related to 4 other human renal cell carcinomas, suggesting that OGF-OGFr interactions dictate the course of cell generation in these tumor cells and was not dependent on a single cell line or characteristics of one tumor.

The use of a tissue culture system in the present studies had the benefit of eliminating confounding problems in *in vivo* experimentation such as 1) immunomodulation, 2) involvement of substances related to neurotransmission (for example, γ -aminobutyric acid, acetylcholine), and/or 3) endocrine or paracrine secretions (for example, pituitary hormones) as mediators of the antitumorigenic effects of opioids. Therefore, one may conclude that opioids act directly on the human renal carcinoma cells to influence growth at the cellular level.

Given evidence that OGF functions in a tissue culture system, one would expect that both the peptide and its receptor would be associated with human renal cell carcinoma. We now report that both elements, peptide and receptor, were found in these cells by immunocytochemistry. Although the OGF receptor has been localized to the nuclear fraction in receptor binding studies,¹⁴ the demonstration of immunoreactivity in the cytoplasm is not inconsistent with these findings because the antibodies to OGF and its receptor are recognizing production in the cytoplasm as well as the putative location proximal to the nuclear envelope. These results are consonant with the findings reported earlier by Zagon and coworkers^{18,19} where OGF and its receptor were noted by immunocytochemistry in other, non-renal tumor cell types. Future studies will have to focus on the characteristics and abundance of the OGF receptor in human renal cell carcinomas to provide more information about the relationship of this receptor to the disease process.

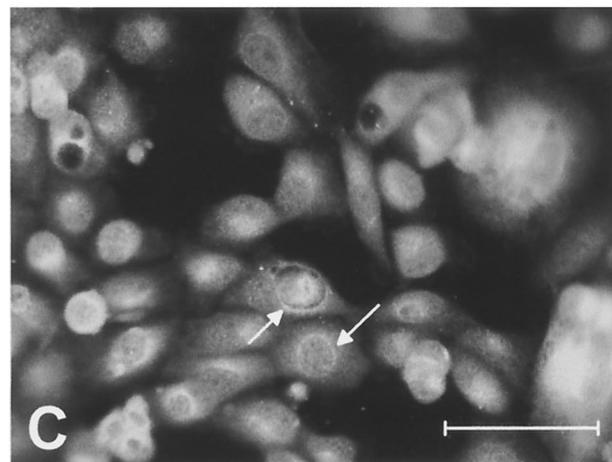
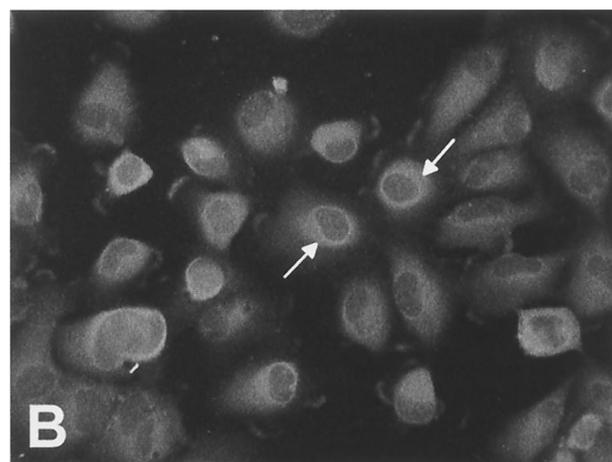
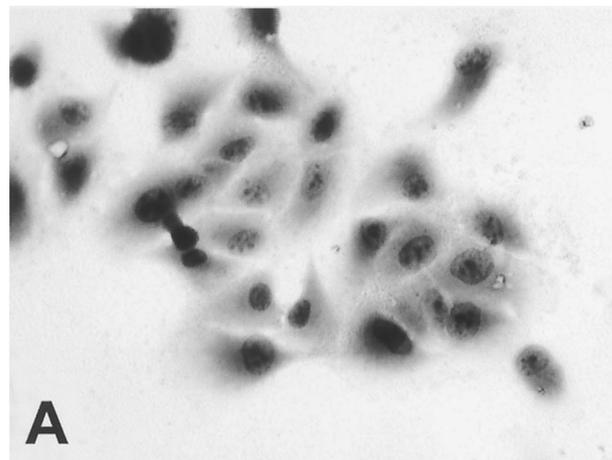


FIG. 8. Photomicrographs of log-phase Caki-2 cells stained with hematoxylin and eosin (A), or antibodies (1:100) to OGF ([Met⁵]-enkephalin) (B), or OGFr (C). Rhodamine conjugated IgG (1:100) served as secondary antibody. Immunoreactivity was associated with cytoplasm (arrows); nucleoplasm was not stained. No immunostaining was detected in cell preparations incubated with antibodies preabsorbed with either excess OGF or the 17 kDa polypeptide of the OGF receptor (data not shown). Scale bar = 440 μ m.

The tonic nature of OGF in tissue culture, with interference of OGF activity using an antibody or opioid antagonist, would infer OGF is found or made in the renal carcinoma cells. OGF would provide the cells with a way to regulate their own reproduction serving as a negative growth factor. Indeed, enough OGF must be manufactured and available to provide some regulation of cellular generation, because interference by a neutralizing antibody or opioid antagonist

results in an enhanced growth pattern. However, because addition of OGF diminishes cellular generation, this would raise the question of whether one mechanism contributing to the proliferation of renal carcinoma cells is a defect (at least partial) in the machinery producing a vital tumor-suppressing element. Although molecular investigations using probes for preproenkephalin (PPE) are needed to confirm the autocrine nature of OGF, studies with clinical specimens will be vital in elucidating the relationship between the abundance of OGF and malignancy.

Estimating the total effect of OGF on the growth of human renal carcinoma cells needs to take into consideration a number of facts. First, the endogenous activity of OGF can be roughly estimated by data on the effects of opioid-receptor blockade using NTX. Specifically, 24 hours after addition of NTX, an increase of 84% from control levels was observed. Second, the capacity of exogenous OGF, presumably acting by saturating sites where endogenous peptide is absent, needs to be given attention. In the case of human renal cell carcinoma, a decrease in cell growth of 29% from control values was recorded within 24 hours of peptide exposure. Thus, summing the effects of endogenous and exogenous OGF activity yields a cumulative effect on growth of up to 113% within a short period of time. Given this profound influence on cell replication, it may be postulated that defects in the transcription and/or translation of OGF would have the effect of decreasing a regulatory element that keeps renal carcinoma cells within a normal framework of replication. Whether exogenous application of OGF would restore or arrest renal carcinoma in the clinical setting is an exciting possibility. Additionally, the integrity of the OGF receptor also must be considered in assessing the etiology and pathogenesis of abnormal human renal cell carcinoma growth. The demonstration of the receptor for OGF by immunocytochemistry herein would permit raising the possibility that these receptors which transduce the signal triggered by OGF to tightly regulate DNA synthesis could be compromised at least in part, resulting in an increase in cell replicative events. And, of course, disturbances of both peptide and receptor would have even more of a deleterious effect by diminishing control mechanisms related to cell generation.

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