

Opioid growth factor receptor (OGFR) expression is downregulated with progression of triple negative breast cancer

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Received July 06, 2015; Revised September 07, 2015; Accepted September 20, 2015; Published Online September 29, 2015

Original Article

Abstract

Purpose: Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that accounts for approximately 15% of the newly diagnosed cancers worldwide, and disproportionately affects younger women and women of color. Although many forms of breast cancer are successfully treated, new therapies are needed for TNBC. A novel regulatory system, the opioid growth factor (OGF) – opioid growth factor receptor (OGFr) axis, plays a determining role in neoplasia. OGF is an endogenous peptide that binds specifically to OGFr to inhibit cell replication. As some human cancers grow, OGFr expression is diminished, thus limiting the therapeutic efficacy of OGF. The OGF-OGFr axis is present in human TNBC cell line MDA-MB-231 and OGF inhibits cell replication in a dosage-related, receptor-mediated manner. **Methods:** The present study investigated whether OGFr protein expression in human breast cancer cell lines grown *in vitro* or transplanted into nude mice, changed with the stage of proliferation or size of tumor using western blotting, semi-quantitative immunohistochemistry, and DNA synthesis techniques. **Results:** Comparison of log and confluent TNBC cultures revealed that OGF expression was significantly decreased in confluent cultures relative to levels in log-phase cells. Western blot analyses confirmed that OGFr was reduced in confluent TNBC and MCF-7 breast cancer cells in comparison to corresponding log-phase cells. Moreover, BrdU labeling was reduced in confluent cells. Small (<500 mm³) and large (>1000 mm³) TNBC tumors grown in nude mice were processed for semiquantitative measurement of OGF and OGFr. The expression of both peptide and receptor in large tumors was downregulated relative to small tumors. **Conclusion:** The reduced expression of the inhibitory peptide and receptor diminishes the efficacy of the OGF-OGFr axis as a biotherapy. These data suggest that the OGF-OGFr pathway is altered with cancer progression and one or more elements of this regulatory pathway may serve as biomarkers for TNBC growth.

Keywords: Opioid Growth Factor; [Met⁵]-Enkephalin; Opioid Growth Factor Receptor; Triple-Negative Breast Cancer

Introduction

Triple negative breast cancer (TNBC) constitutes less than 20% of all cases of invasive breast cancer, but remains the most aggressive form of breast cancer.^{1,2} TNBC does not express the three hormonal receptors estrogen (ER), progesterone (PR), and human epidermal growth factor (HER2) that serve as targets for chemotherapy and immunotherapy for other breast cancers,^{3,4} thus making it resistant to many of the viable treatment options available for breast cancer therapy.^{2,5-7} The prognosis for successful treatment and extended survival for women with ER, PR, and/or HER2 detected in breast tissue approaches 98%,² whereas the median survival time for patients with TNBC is 13 months.^{2,7} TNBC also has an increased prevalence in younger females, women of color, and those from lower socioeconomic countries.² A few studies have reported new agents to treat TNBC,⁷⁻⁹ but there remains an unmet medical need to study the underlying biology of TNBC.

Previous studies in our laboratory investigated the presence and function of a novel biological pathway in TNBC.¹⁰ The opioid growth factor (OGF) – OGF receptor (OGFr) pathway is present in a wide variety of cancers,¹¹ including TNBC, and has been shown to modulate neoplasias related to the female reproductive system.¹²⁻¹⁶ The OGF-OGFr axis inhibits the replication of human ovarian cancer cells in culture and tumor growth in nude mice.¹²⁻¹⁶ OGF, chemically termed [Met⁵]-enkephalin, is a constitutively-expressed pentapeptide that inhibits cell proliferation by stimulating the cyclin-dependent inhibitory kinase pathway and slowing the transition between the G₁ and S phases of the cell cycle.^{17,18} The mechanism of action of OGF is to inhibit DNA synthesis by upregulating cyclin-dependent inhibitory kinases.¹⁹ In squamous cell carcinoma of the head and neck, p16 is upregulated in response to OGF treatment,²⁰ whereas p21 is upregulated in human pancreatic cancer.²¹ Other kinases such as p15 and p18 were not affected by OGF

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Cite this article as: Worley B, Zagon I, McLaughlin P. Opioid growth factor receptor (OGFR) expression is downregulated with progression of triple negative breast cancer. *Int J Cancer Ther Oncol* 2015; 3(4):347. DOI: 10.14319/ijcto.34.7

exposure. *In vitro* studies examining two TNBC cell lines, MDA-MB-231 and BT-20, revealed the presence of both OGF peptide and OGFr by immunohistochemical staining of log-phase cells.¹⁰ Specificity of peptide and receptor were confirmed by antibody neutralization and molecular studies to knockdown classical opioid receptor proteins. OGF treatment inhibited TNBC cells in a dosage related, receptor mediated, and reversible manner. Based on siRNA studies, OGFr was the specific receptor involved in the mediation of the inhibitory effects of OGF. These data confirmed earlier studies reporting that the OGF-OGFr axis mediates *in vitro* proliferation in MDA-MB-231 and MCF-7 human breast cancer cell lines, with no changes in growth following OGF treatment if OGFr expression was reduced.^{10,11}

Analysis of clinical samples from patients with squamous cell carcinoma of the head and neck demonstrated that late stage tumors had markedly fewer OGF receptors relative to benign margins or normal tonsil tissue based on binding assays and western blot analyses, suggesting that OGFr protein expression, but not RNA, became defective as tumor growth progressed.²² Preclinical studies on human squamous cell carcinoma of the head and neck inoculated into nude mice revealed that OGFr expression normalized for protein was diminished in larger tumors relative to smaller tumors.²³ Other clinical studies involving resected ovarian tumors demonstrated that malignant tissue had significantly less OGFr expression relative to benign ovarian cysts and ovarian surface epithelial cells.¹⁵ Analysis of tissue samples from cysts and late stage (III/IV) tumors revealed a decrease in OGF expression by 29% and 58%, respectively, from normal cells.¹⁵ Expression of OGFr was decreased by 34% and 48% in cysts and tumors, respectively, relative to normal levels. Receptor binding assays on cysts and malignant tumor tissues indicated that tumors had 5.4-fold fewer OGF receptors than cysts, suggesting that deficits in the OGF-OGFr axis are amplified as cancer progresses.¹⁵

The present study examined the hypothesis that progression of TNBC may be associated with a decrease in OGFr protein rendering the tumor tissue less responsive to endogenous or exogenous OGF treatment. Utilizing both *in vitro* and *in vivo* models of MDA-MB-231 human TNBC, OGFr protein expression in log and confluent cells, as well as small and large tumors grown in nude mice, was evaluated by immunohistochemistry and western blot analyses. For comparison, human breast cancer cell line MCF-7 that contains estrogen and progesterone receptors was evaluated for OGFr expression in log and confluent phases of growth. Both breast cancer cell lines were treated with OGF to determine the efficacy of this novel biotherapy in highly proliferating cells (i.e., log phase) and in a setting of reduced replication (i.e., confluence).

Methods and Materials

Cell culture and cell growth assays

The human breast cancer lines MDA-MB-231 and MCF-7 were purchased and verified by The American Type Culture Collection (Manassas, VA). MDA-MB-231 cells lack estrogen, progesterone, and human epidermal growth factor receptor 2 (HER2) receptors, designating these cells as a TNBC cell line.¹ This cell line is derived from a 51-year-old female with metastatic breast cancer. MCF-7 cells were derived from a 69-year-old Caucasian female and characterized at the Michigan Cancer Foundation to contain estrogen and progesterone receptors.²⁴ MDA-MB-231 cells were grown in Leibovitz L-15 medium supplemented with 2 mM L-glutamine, and MCF-7 cells were cultured in Dulbecco's modified Eagle's medium. All media were supplemented with 10% fetal calf serum, 1.2% sodium bicarbonate, and antibiotics (5,000 Units/ml penicillin, 5 mg/ml streptomycin). Cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C.

For growth assays, cells were plated in 24-well plates and counted 24 hours later (time 0) to determine seeding efficiency. In some assays, 10⁻⁶ M OGF or sterile water (equivalent volume, 1 µl) was added at time 0; media and compounds were changed daily.¹² All drugs were prepared in sterile water and dilutions represent final concentrations. Cells were harvested at designated times and counted by hemacytometer using trypan blue exclusion methodology. At least two aliquots per well from two wells/treatment at each time point were sampled.

Animals and tumor growth

Female 4- to 5-week old athymic *nu/nu* mice were purchased from Charles River Laboratories (Wilmington, MA) and housed in pathogen-free isolators in a controlled-temperature room (22-25°C) with a 12/12 hour light/dark cycle. Sterile rodent diet and water were available *ad libitum*. All procedures were approved by the IACUC committee of the Pennsylvania State University College of Medicine. Following a 48 hour acclimation period, unanesthetized mice were inoculated subcutaneously in the right scapular and left flank regions with MDA-MB-231 cells (8 × 10⁶ cells/0.1 ml per injection). Mice were weighed weekly and observed daily for the appearance of tumors. Individual tumors were measured three times/week using a digital vernier caliper and volumes calculated as previously described.^{13,23,25}

Tissue collection

Tumors were harvested as small or large when the tumor volume reached approximately 350 mm³ or 1000 mm³, respectively. Tumor bearing mice were euthanized with an injection (0.1 ml/mouse) of Euthasol (Virbac AH, Inc, Fort Worth, TX) followed by cervical dislocation; the body cavity

was examined for metastases.^{13,16} Tumor tissue was either frozen in isopentane chilled on dry ice for immunohistochemistry or placed in RIPA buffer and stored at -20° C for western blotting protein assays.¹⁶ All tissues were processed within 2 weeks of collection.

Immunohistochemistry

The presence and quantity of OGF and OGF α were evaluated in both cells and tumor tissue following published protocols.^{10,11,16} Log-phase (10⁴ cells/well) and super confluent (10⁶ cells/well) MDA-MB-231 and MCF-7 cells were seeded onto 22 × 22 mm glass coverslips in 6-well plates. After 72 hours in culture the cells were fixed and permeabilized in 95% ethanol and acetone at -20° C, rinsed in Sorenson's phosphate buffer (SPB), and blocked with SPB containing 1% normal goat serum and 0.1% Triton-X 100 at room temperature for 15 min. Cells were stained 1:200 with anti-OGF-IgG (CO172) and anti-OGF α -IgG (BO344) polyclonal antibodies diluted in SPB containing 1% normal goat serum and 0.1% Triton-X 100 for 2 hours at room temperature, followed by secondary goat anti-rabbit IgG antibodies (1:1000) for 45 min.^{16,25} Cells incubated with secondary antibody only were considered controls.

Tumor tissue was cryosectioned (10 μ m) and stained as described previously with primary antibody for 18 hours at room temperature, followed by secondary antibody.^{12,13,15}

Mean intensity of positively stained tumor cells was determined from at least 8 fields per coverslip and 3 coverslips/group.¹² With regard to tumors, random samples of 3 fields/section from 6 sections/tumor and 2 tumors/group were assessed.^{13,15} All specimens were visualized with an Olympus IX-81 epifluorescence microscope.

Protein isolation and western blotting

OGF α expression levels were evaluated in cells and tumor tissue by western blotting.^{10,12-15} Cells were sheared 3-4 times with a 25 gauge needle while tumor tissue was homogenized in RIPA buffer containing PMSF protease inhibitors. Equal amounts of protein (40 μ g) were subjected to SDS polyacrylamide gel electrophoresis; nitrocellulose membranes were probed with goat polyclonal IgG antibody to OGF α (1:500) and donkey anti-goat IgG antibody (1:8000). Densitometric assessment of bands was normalized to GAPDH.

DNA synthesis

OGF inhibition of DNA synthesis was evaluated in cell culture using 5-bromo-2'-deoxyuridine (BrdU) incorporation. Log-phase and confluent MDA-MB-231 and

MCF-7 cells were seeded onto 22 × 22 mm glass coverslips in 6-well plates and treated with 0.2 μ l OGF or sterile saline daily for 72 hours; media and drugs were changed daily. Three hours prior to fixation, 30 mM BrdU (Sigma Chemicals, Indianapolis, IN) was added to each well. Cells were rinsed, fixed in 1:1 methanol acetone for 20 min at 4° C, and stained with anti-BrdU mouse monoclonal antibody (1:200). At least 1000 cells/treatment from 2-4 coverslips/treatment were counted. Labeling indexes were calculated as the number of positively stained cells divided by the total number of cells.

Data analyses

All data were analyzed using two-tailed Student's *t*-test for two groups, or analysis of variance with subsequent comparisons made with Newman-Keuls tests for multi-group comparisons. All analyses were conducted with GraphPad Prism 5 software (GraphPad Inc, San Diego, CA). *P*-values less than 0.05 were considered statistically significant.

Results

Presence and relative expression of OGF and OGF α in human breast cancer cells

MDA-MB-231 TNBC cells grown to either log phase or confluency were stained with OGF and OGF α antibodies (**Figure 1A**). Semi-quantitative densitometric assessment of peptide and receptor expression revealed a 22% decline in receptor expression (anti-BO344) and a 10% reduction in OGF (anti-CO172) peptide staining in confluent cell cultures relative to expression levels in log-phase cells (**Figure 1B**). However, exogenous OGF treatment of log-phase MDA-MB-231 cells exhibited significant reductions in cell number after 72 and 96 hours of peptide exposure relative to sterile water-treated cells, suggesting that the OGF-OGF α axis was functional in TNBC (**Figure 1C**).

For comparison, MCF-7 human breast cancer cells containing estrogen and progesterone receptors were grown to log phase and confluency, and immunostained with anti-OGF and anti-OGF α antibodies (**Figure 2A**). Assessment of peptide and receptor expression in these breast cancer cells revealed a 33% reduction in receptor and 29% reduction in peptide levels in confluent cultures relative to log-phase cells (**Figure 2B**). Exogenous treatment of log-phase MCF-7 cells with OGF demonstrated growth inhibition at 48 and 72 hours relative to cells treated with sterile water (**Figure 2C**), suggesting that the OGF α was intact and functioning.

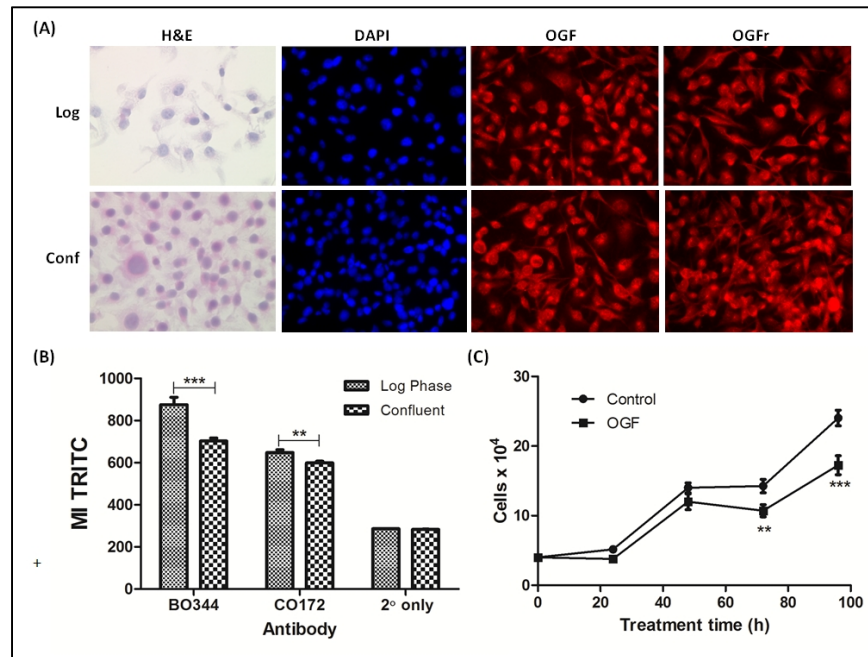


FIG. 1: The presence and function of the OGF-OGFr axis in human triple negative breast cancer cells. (A) Expression of OGF and OGFr in log-phase and confluent MDA-MB-231 cells as detected by immunocytochemistry. Cultures were stained with antibodies to OGF (CO172, 1:200) or OGFr (BO344, 1:200); cells stained with secondary antibody served as controls; (B) Data (mean \pm SEM) represent the mean intensity of TRITC staining with BO344 and CO172 and were analyzed individually using *t*-tests. Significantly different from respective log-phase cells at *** $P < 0.001$ and ** $P < 0.01$; (C) OGF inhibition of cells growth. MDA-MB-231 cells were treated with 10^{-6} M OGF or sterile water. Cells were counted at 24, 48, 72, and 96 hours. Data represent means \pm SEM for triplicate samples. Significantly different from control time points at ** $P < 0.01$ or *** $P < 0.001$.

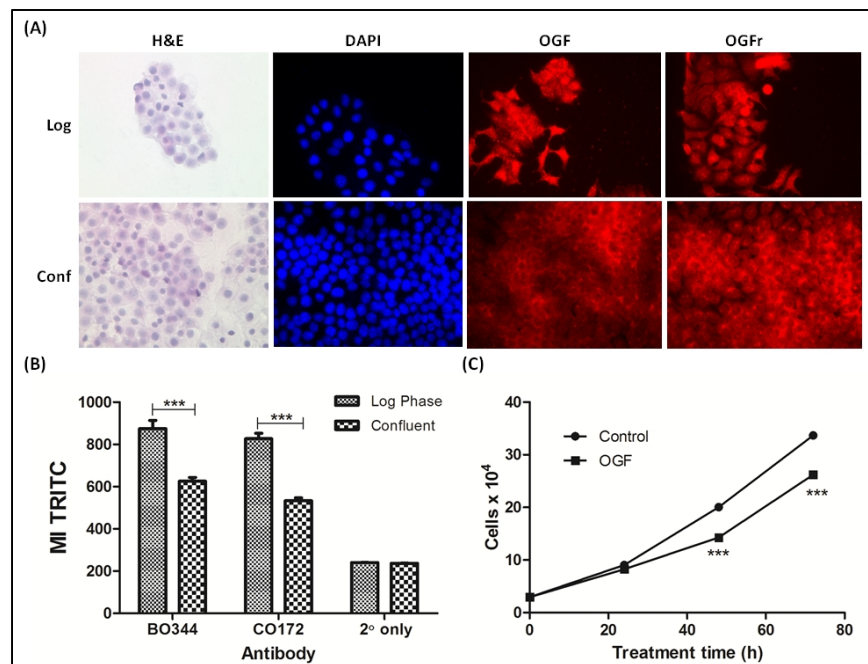


FIG. 2: The presence and function of the OGF-OGFr axis in MCF-7 human breast cancer cells. (A) Expression of OGF and OGFr in log-phase and confluent MCF-7 cells as determined by immunocytochemistry. Cultures were stained with antibodies to OGF (CO172, 1:200) or OGFr (BO344, 1:200); cells stained with secondary antibody only served as controls; (B) Data (mean \pm SEM) represent the mean intensity of TRITC with BO344 and CO172 and were analyzed individually using *t*-tests. Log-phase cells significantly different from confluent cells at *** $P < 0.001$; (C) OGF inhibition of cell growth. MCF-7 cells were treated with 10^{-6} M OGF or sterile water. Cells were counted at 24, 48, or 72 hours. Data represent means \pm SEM for triplicate samples. Significantly different from control time points at *** $P < 0.001$.

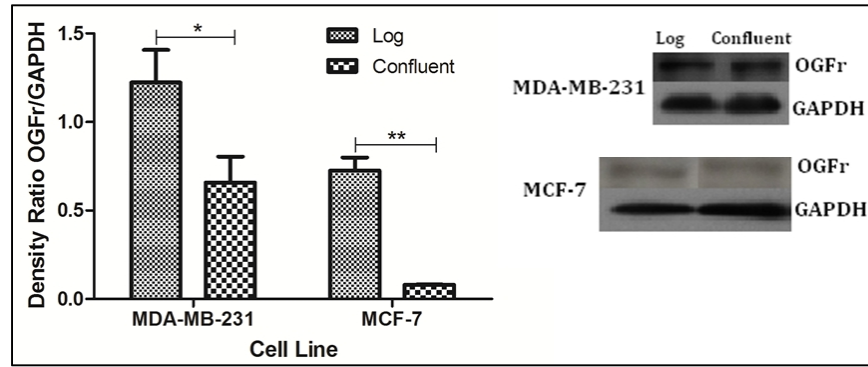


FIG. 3: Western blots of OGFr protein expression in MDA-MB-231 human TNBC and MCF-7 breast cancer cells. Histograms represent densitometric ratios of OGFr/GAPDH (means \pm SEM) from 3 blots. OGFr expression differs from log-phase and confluent cells at $*P < 0.05$ or $**P < 0.01$.

Imaging of the immunocytochemical preparations revealed that the cellular distribution patterns of OGF and OGFr within the cancer cells were comparable for both breast cancer cell lines.

Protein expression of OGFr as evaluated by western blot analysis, and normalized to GAPDH, revealed that receptor expression was markedly diminished in confluent cells relative to log-phase cultures (Figure 3). Nuclear homogenates of log-phase and confluent cultures of both MDA-MB-231 TNBC and MCF-7 breast cancer cells also displayed a significant decrease in receptor expression in confluent cultures in comparison to those values from log-phase cells. Confluent TNBC cells had a 50% reduction in receptor expression relative to log-phase cells, whereas confluent MCF-7 cells had 86% less expression of OGFr in comparison to log-phase cells.

DNA synthesis rates in log phase and confluent cultures of TNBC or MCF-7 breast cancer cells

To evaluate whether the mechanism underlying the inhibitory effects of OGF was altered, DNA synthesis as measured by BrdU incorporation was assessed in log and confluent cultures treated with OGF or sterile water

(controls). In control cultures of both TNBC (Figure 4A) and MCF-7 human breast cancer (Figure 4B), BrdU incorporation was greatest in log-phase cells relative to confluent cultures. OGF-treated log and confluent phase MDA-MB-231 TNBC cells exhibited a 44% and 29.8% decrease, respectively, in BrdU incorporation relative to control treated TNBC cells; these reductions in DNA synthesis were comparable. Evaluation of cultures of MCF7 breast cancer cells treated with OGF revealed a 30% decline in DNA synthesis in log-phase cells, and a 77% reduction in BrdU incorporation in confluent cultures relative to that recorded in sterile-water treated confluent cultures (Figure 4).

Calculation of OGF inhibition of growth over a 72-hour period of time revealed that log-phase MDA-MB-231 cells were inhibited 35% relative to controls. In comparison, growth of confluent TNBC cells was reduced approximately 15%. Reductions in cell replication of log-phase MCF-7 cells ranged between 28 and 32% relative to 12% reductions in confluent cells following OGF treatment. These data suggest that confluent cells are less responsive to OGF treatment than log-phase cells, supporting the integral role of OGFr to mediate growth inhibition by OGF.

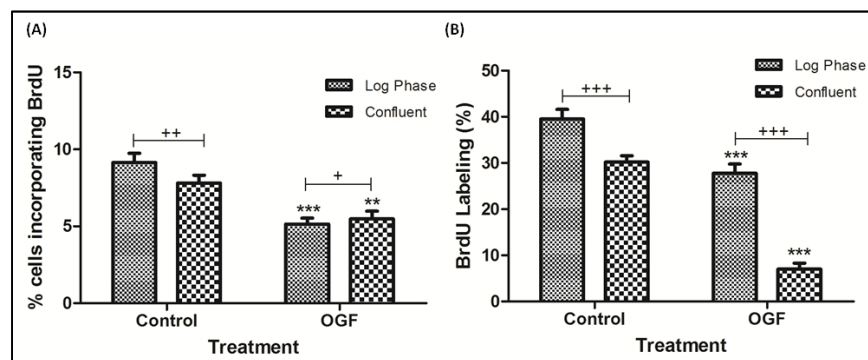


FIG. 4: The inhibitory effect of OGF on DNA synthesis. MDA-MB-231 cells (A) and MCF-7 cells (B) were seeded on 22 mm coverslips and treated for 3 hr with OGF (10^{-6} M) or sterile water (control) and 30 μ m BrdU. Data (mean \pm SEM) represent the percent of BrdU-positive cells. Significantly different from respective control treated cells at $**P < 0.01$ or $***P < 0.001$, and from respective log-phase cells at $+P < 0.05$, $++P < 0.01$, or $+++P < 0.001$.

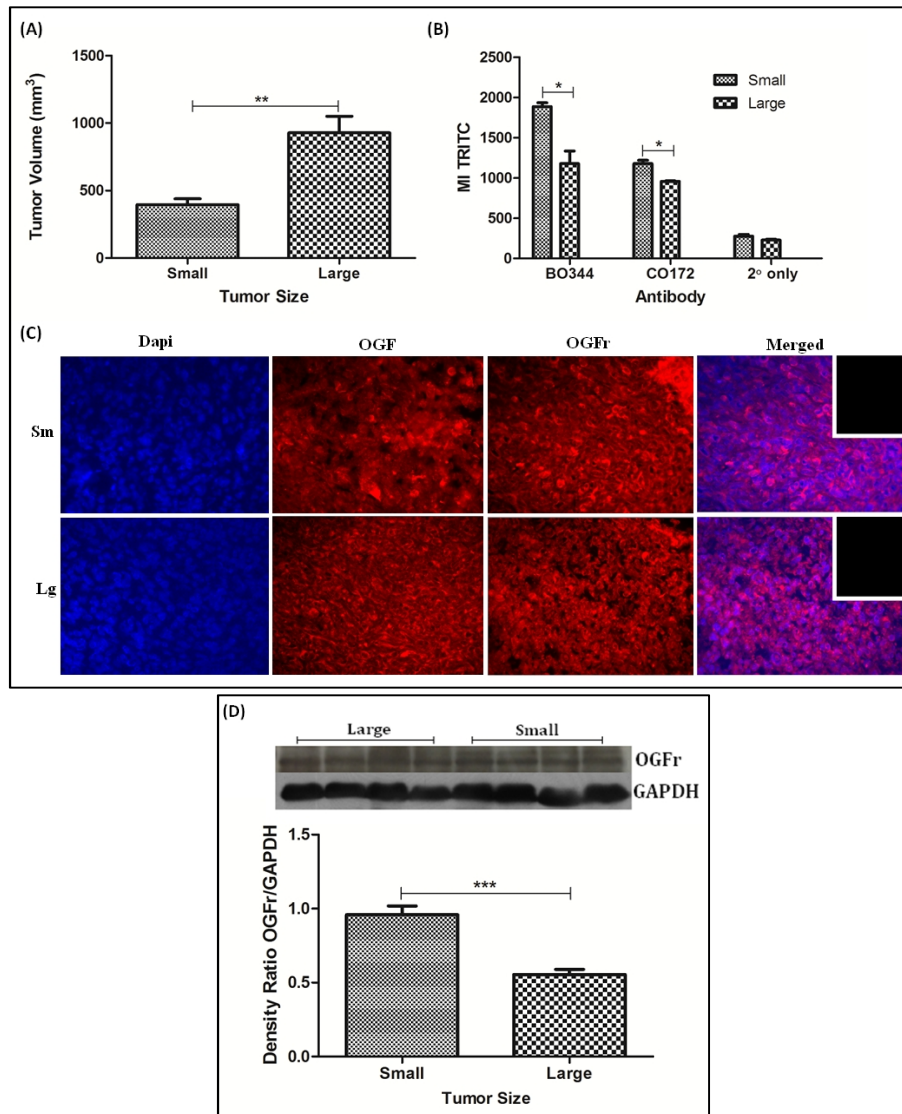


FIG. 5: The presence of the OGF-OGFr axis in nude mouse tumors of human breast cancer as determined by immunocytochemistry. (A) Large and small tumor volumes differed significantly at $**P < 0.01$; (B, C) Differential expression of OGF and OGFr in large and small tumors is detected by immunohistochemical staining (C) and semi-quantitative analyses. Tumors were sectioned and stained with antibodies to OGFr (BO344, 1:200) and OGF (CO172, 1:200); sections stained with secondary antibody only served as controls. (A) Data (mean \pm SEM) represent mean intensity of TRITC with BO344 (OGFr) and CO172 (OGF). Large tumors differed significantly from respective small tumors at $**P < 0.01$ using two-tailed *t*-test analyses. (D) Western blots of OGFr protein expression in small and large tumors; GAPDH staining served to control for protein load. Histogram represents the densitometric ratio (means \pm SEM) from 3 blots. OGFr expression differed significantly between small and large tumors at $***P < 0.001$.

TNBC tumor growth in nude mice

Female athymic nude mice weighed approximately 20 g at the beginning of experimentation, and no differences in body weight were noted in mice with small or large tumors at the time of termination. No metastases were noted. Tumor weight and tumor volume of small and large TNBC tumors differed significantly (**Figure 5A**). Tumor volumes were less than 500 mm³ for small tumors whereas large tumors were greater than 1000 mm³.

OGFr immunolabeling was detected in the cytoplasm and nucleus of specimens from small and large TNBC tumors

(**Figure 5B, C**); no immunoreactivity could be observed in sections processed with secondary antibody only. Semi-quantitative densitometry revealed that receptor expression was reduced 37% in the large tumors, and 15% for OGF peptide expression, relative to small tumors (**Figure 5C**).

Isolated OGFr protein was evaluated by western blot analyses and was reduced by 50% in large tumors relative to small tumors (**Figure 5D**).

Discussion

These studies are the first to report that the presence of the OGF-OGFr regulatory axis in TNBC provides a novel pathway for controlling the progression of neoplasia, and that OGFr expression is downregulated as TNBC cells become confluent or tumors grow larger. Together these data support and extend our observations that an intact OGF-OGFr pathway plays an active role in modulation of neoplasia, and that OGFr is required for modulating tumor growth by the inhibitory peptide OGF.¹⁰⁻¹⁵

Several lines of investigation support this hypothesis. Utilizing comparison between log-phase highly proliferative cells and those that are confluent, OGFr expression in two human breast cancer cell lines was markedly reduced in confluent cells, suggesting that as neoplastic cells become more differentiated, the receptor protein is downregulated in both immunostaining and western blotting. Secondly, in culture, endogenous OGFr expression is reduced in confluent cells relative to log-phase TNBC or MCF-7 cells. Although OGF continued to be effective at suppressing replication suggesting that the remaining OGFr receptors were functional, the magnitude of growth inhibition by OGF was reduced in confluent cultures relative to that in log-phase cells. Levels of DNA synthesis as measured by BrdU incorporation confirmed this observation.

In vivo comparison of small and large tumors, which is not completely analogous to human cancer growth, but does provide a model for comparison within a reasonable period of time, confirmed and extended the tissue culture observations. OGF and OGFr expression was diminished relative to that recorded in log-phase cells and small tumors. Up to 50% reductions in receptor expression were recorded. Using tumors that were generally 2-fold greater in diameter and volume for comparison, small tumors contained OGFr receptors that had 2-fold more receptors than large tumors. Likewise, evaluation of the MCF-7 breast cancer cell line that contains estrogen and progesterone receptors, revealed the loss of OGFr expression in confluent cultures relative to log-phase cells.

Given that TNBC is an aggressively growing breast cancer, the downregulation of a regulatory pathway in advanced stage tumors may serve as a potential biomarker for cancer progression and response to therapy.

OGFr must be present for exogenous biotherapy (i.e., OGF infusion) to be effective. The specific receptor modulating the inhibitory effects of OGF is reported to be OGFr,²⁸ and breakdown or loss of the receptor limits the inhibitory efficacy of OGF.^{11,14} Disruption of the OGF-OGFr axis in human cancer cell lines by continuous exposure to an opioid antagonist, neutralization of endogenous opioids, or knockdown of the OGFr stimulates DNA synthesis.³⁻⁵ Thus

biological loss of the receptor in advanced stages of neoplasia would be detrimental for natural regulation by endogenous opioids such as OGF.

Previous research has documented the presence of the OGF-OGFr axis in a wide variety of human cancers, including pancreatic, colon, renal, ovarian, neuroblastoma, hepatocellular adenoma, SCCHN, and breast.^{11,17} These data for TNBC are comparable to that reported for squamous cell carcinoma of the head and neck (SCCHN). In an earlier study in nude mice, small sized SCCHN tumors had 3- to 7-fold more OGFr than large tumors. However, binding affinity of receptors from small or large tumors were unchanged in the SCCHN tumors.²³ Disruption of the OGF-OGFr pathway can occur by disease, genetic mutation, continuous exposure to opioid antagonists, neutralization of endogenous opioids, or other imbalances that can accelerate neoplastic cell growth.¹⁷ Conversely, upregulation of the OGF-OGFr axis has been shown to repress cell division in both normal and abnormal cells.^{26,27}

TNBC has received minimal attention for specific treatment modalities targeting its unique biology. Most current approaches are still in the preclinical stage,²⁸ but warrant follow-up trials. The clinical significance of data in this report lies in the fact that OGF is an endogenous opioid that can be safely infused for therapy. Moreover, low dosages of naltrexone (LDN) provide a second approach for upregulating the OGF-OGFr axis in TNBC patients. LDN is an oral medication that has been shown to be safe over long periods of time for treatment of autoimmune diseases.²⁹ However, preclinical studies with LDN and human breast cancer cell lines¹⁰ remain promising for the role of the OGF-OGFr axis in modulation of TNBC. In all cases, OGFr is the specific receptor mediating the inhibitory growth effects of OGF or LDN. Loss of function related to defective or downregulated protein expression of OGFr would render these therapies ineffective. Knowledge of this biomarker may be useful for individualized patient therapy of TNBC.

Conclusion

TNBC accounts for approximately 15% of all cases of invasive breast cancer, but does not respond to most current therapies.^{6,7} TNBC frequently affects younger women and women of color, and is characterized by a high tendency to metastasize outside the breast.^{7,8} Despite a better than 98% five-year relative survival rate for women with successfully treated breast cancer containing estrogen and progesterone receptors, the median survival rate for patients with metastatic TNBC is only 13 months.⁷ There is an unmet medical need to develop novel treatments that target underlying biology of tumor growth. These data confirm that OGF can be used to inhibit the proliferation of human TNBC, as well as other breast cancers *in vitro*, demonstrating

the potential of the OGF-OGFr pathway as a successful biological therapy for treatment of TNBC.

Conflict of interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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