Thomas Jefferson University

Annual Progress Report: 2008 Formula Grant

Reporting Period

July 1, 2012 – December 31, 2012

Formula Grant Overview

The Thomas Jefferson University received $3,455,597 in formula funds for the grant award period January 1, 2009 through December 31, 2012. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Role and Regulation of Focal Adhesion Kinase in Melanoma - Malignant melanoma is the deadliest form of skin cancer. Currently, the spreading of melanoma, known as metastasis, is only preventable by early detection and surgical excision of primary tumors. Therefore, it is critical that we understand the mechanisms underlying melanoma initiation and progression. The depth of invasion of a melanoma into the skin (dermis) is a determinant of treatment outcome. A gene, labeled B-RAF, is mutated in two-thirds of melanomas. This project has identified a target of the B-RAF gene known as focal adhesion kinase (FAK). This study will test whether B-RAF regulates FAK, which in turn promotes melanoma cell invasion into the dermis. Ultimately, the focus of this project is to identify why melanomas display invasive growth and, in doing so, identify novel targets for therapeutic intervention.

Duration of Project

1/1/2009 – 06/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 2: Project Title and Purpose

Prolactin and Growth Factor Signaling in Breast Cancer - Progression of breast cancer from early solitary lesions to advanced metastatic disease requires loss of cellular differentiation and epithelial-to-mesenchymal transition (EMT). While extensive efforts have been devoted to understanding the factors that allow this transition to occur in breast cancer, little work has focused on factors that prevent EMT, or on molecular strategies to reverse EMT for use in differentiation therapy. The long-range goal of this laboratory is to identify molecular
mechanisms that govern growth and cellular differentiation of human breast cancer to improve therapies, and to use this knowledge to improve drug responsiveness of individual tumors, so that more effective, personalized medical treatment can be realized for cancer patients.

**Duration of Project**

1/1/2009 - 12/31/2012

**Project Overview**

The central hypothesis is that the prolactin receptor (PRLR)-Jak2-Stat5 pathway promotes cellular differentiation and suppresses epithelial-to-mesenchymal transition (EMT), loss of estrogen receptor (ER) expression, and drug resistance of luminal breast cancer.

Aim #1 Determine whether the PRLR-Jak2-Stat5 pathway in human breast cancer is inhibited by EMT-promoting growth factors in the tumor microenvironment. The hypothesis is that paracrine growth factor receptor activation by epidermal growth factor (EGF) ligands inhibits the differentiation-promoting PRL-Jak2-Stat5 pathway in breast cancer, through a mechanism that involves Erk1/2 phosphorylation of Jak2 on the inhibitory Ser523 residue. We will a) determine whether experimental EGFR/Her2-mediated signaling inactivates PRL-induced Stat5 activation in breast cancer lines through Jak2 inhibition, due to activation of either Erk1/2, Jnk and/or Akt pathways, and b) analyze whether signaling by EGF receptors and hyperactivation of Erk1/2, Jnk and/or Akt kinases is associated with inactivation of Stat5 in two large sets of human breast cancer specimens (n=850).

Aim #2 Determine whether Stat5α activation is sufficient to overcome dedifferentiation, EMT, and loss of ERα induced by EGFR/Her2 in human breast cancer. The hypothesis is that EGFR/Her2 inhibition of the PRLR-Jak2-Stat5 pathway is required for EGFR/Her2-induced EMT, cell-cycle progression, and loss of ERα expression. We will determine a) whether Stat5 reactivation overcomes EGFR/Her2-induced EMT, and b) whether Stat5 reactivation restores ERα expression in ERα-negative, EGFR/Her2-positive breast cancer in vitro and in vivo.

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Expected Research Outcomes and Benefits

More than one million new cases of breast cancer are diagnosed each year worldwide, and an estimated 370,000 women die each year. The majority of breast cancer fatalities are caused by metastasis (spreading to other organs) of the primary tumor. Identifying the molecular changes that lead to metastatic progression is critical for developing better strategies to prevent spreading and treat advanced breast cancer. While it is generally accepted that loss of differentiation and epithelial-to-mesenchymal transition (EMT) are required for breast cancer metastasis, efforts to reverse de-differentiation of breast cancer have been limited.

The Stat5 protein is a suppressor of human breast cancer dedifferentiation, invasion, and progression. Studies are also designed to determine whether pharmacologic inhibition of EGFR/ErbB2 will restore prolactin (PRL)-Jak2-Stat5a/b signaling in breast cancer in vitro and in vivo. This is important because PRL is a key activator of Stat5 in normal and malignant breast epithelia. If our hypotheses are correct, EGFR/Her2 inhibition combined with Stat5 hyperactivation (e.g., by prolactin or Stat5-ptpase inhibitors) plus antiestrogens may be effective against aggressive ERα-negative, EGFR/Her-2 positive breast cancer.

Furthermore, combined biomarker analyses of Stat5, ERα and EGFR/Her-2 are expected to improve diagnostic classification of human breast cancer and prediction of response to targeted therapies. More effective personalized medicine for breast cancer patients could be a result. Finally, the scientific environment at the Kimmel Cancer Center at Thomas Jefferson University is highly collaborative and the possibilities for translational research are outstanding, so that if successful, the project can move forward with clinical trials.

Summary of Research Completed

We are pleased to report continued solid progress during year 4 of this project.

*Novel Progress showing that prolactin maintains differentiation and drug sensitivity of luminal breast cancer by suppressing progestin-induced expansion of the CK5-positive tumor-initiating cell population.*

Research related to both Aim 1 and Aim 2 completed during the fourth fiscal year has resulted in novel discoveries. Prolactin controls the development and function of milk-producing breast epithelia but also supports growth and differentiation of breast cancer, especially luminal subtypes. A principal signaling mediator of prolactin, Stat5, promotes cellular differentiation of breast cancer cells in vitro, and loss of active Stat5 in tumors is associated with anti-estrogen therapy failure in patients. In luminal breast cancer progesterone induces a cytokeratin-5 (CK5)-positive basal cell-like population. This population possesses characteristics of tumor stem cells including quiescence, therapy-resistance, and tumor-initiating capacity. Here we report that prolactin counteracts induction of the CK5-positive population by the synthetic progestin R5020 (Pg) in luminal breast cancer cells both in vitro and in vivo. CK5-positive cells were chemoresistant as determined by four-fold reduced rate of apoptosis following docetaxel exposure. Pg-induction of CK5 was preceded by marked up-regulation of BCL6, an oncogene and transcriptional repressor critical for the maintenance of leukemia-initiating cells.
Knockdown of BCL6 prevented induction of CK5-positive cell population by Pg. Prolactin suppressed Pg-induced BCL6 through Jak2-Stat5 but not Erk- or Akt-dependent pathways. Clinical relevance of these findings is suggested by a positive correlation between levels of BCL6 and CK5 protein in hormone receptor-positive breast cancer among premenopausal but not postmenopausal patients, and high levels of BCL6 or CK5 correlated with unfavorable outcome. Suppression of Pg-induced CK5-positive cells represents a novel pro-differentiation effect of prolactin in breast cancer. These insights may have direct implications for breast cancer progression and therapy since loss of prolactin receptor signaling is frequent and BCL6 inhibitors are emerging for lymphoma treatment. The key results from this progress are provided below.
Figure 1. Prolactin suppresses CK5 mRNA and protein levels in human breast cancer cells. 
(A) qRT-PCR analysis of CK5 mRNA extracted from T47D xenograft tumors in mice treated with either vehicle or prolactin for 48 h. Individual values are plotted and horizontal markers indicate median levels (P=0.001 by Mann-Whitney U test). (B) qRT-PCR (top) and immunoblot (bottom) analysis of CK5 mRNA and protein levels, respectively, in extracts of cultured T47D treated with vehicle (Control), Prolactin (PRL), β-Estradiol (E2), or R5020 (Pg) for 48 h. (C) Immunocytochemistry using DAB chromogen (brown) for CK5 and hematoxylin counterstain of sections of formalin-fixed, paraffin-embedded pellets of T47D cells treated with hormones as indicated for 24 h. Representative images are shown (top) with quantification for cellular CK5-positivity plotted (bottom). In Pg-treated cells, the percentage of CK5-positive cells was 3.8 times higher (95% CI: 1.6, 8.8, p=0.005) as compared to prolactin+Pg-treated cells.
Figure 2. Prolactin suppresses the fraction of chemoresistant Pg-induced CK5-positive cells. (A) Representative images of multiplexed immunocytochemistry of T47D cell cultures treated with vehicle (Control), Prolactin (PRL), β-Estradiol (E2), or R5020 (Pg) for 24 h before addition of docetaxel (Dx) or vehicle for 48 h and stained for CK5 (red), cleaved caspase-3 (green), and DAPI (blue). (B) Bar graph of percent of CK5-negative (blue bars) or CK5-positive (red bars) cells that were positive for cleaved caspase-3. Quantification revealed a four-fold reduced rate of cleaved caspase-3 staining in CK5-positive cells than in CK5-negative cells (odds ratio 0.25, 95% CI: 0.11, 0.56, P<0.001) (C) Overall percentage of cells positive for CK5 in the cultures shown in panel A. Odds of CK5 positivity were 50% lower for cells treated with prolactin plus Pg (5.7% CK5+) than for cells exposed to Pg alone (10.3% CK5+) (odds ratio= 0.50, 95% CI: 0.39, 0.64, p<0.001).
Figure 3. Progestin induces BCL6 expression and prolactin suppresses progestin-induced BCL6. 
(A) Immunoblots of BCL6, CK5, and GAPDH protein levels of T47D cells treated with the synthetic progestin R5020 (Pg) at the indicated doses for 24 h (top), or the indicated times with 20nM R5020 (Pg) (bottom). (B) Immunoblots of BCL6 protein in extracts of T47D cells treated with vehicle (Cntrl), Prolactin (PRL), β-Estradiol (E2), or R5020 (Pg) for the indicated times. 
(C) Immunoblots of BCL6, CK5, and GAPDH protein levels in extracts of T47D cells treated with hormones in the presence or absence of the progesterone receptor antagonist RU486 for 48 h. 
(D) Immunoblots of BCL6, phosphorylated-Stat5 (p-Stat5), and GAPDH protein levels in extracts of T47D, MCF-7, ZR75-1, BT-474, and HCC1937 cells treated with hormones for 72 h.
Figure 4. Progestin induction of CK5 requires induction of BCL6. (A) Immunoblot of BCL6, CK5 and GAPDH in extracts of T47D and BT-474 cultures treated with non-target shRNA (NTC) or one of two independent BCL6 shRNAs (G2 and F11) for 48 h, followed by addition of vehicle (Cntrl), Prolactin (PRL), β-Estradiol (E2), or R5020 (Pg) for 48 h. (B) Quantification of CK5 immunocytochemistry (ICC) of T47D cultures exposed to either NTC shRNA or BCL6 shRNAs G2 or F11. Percent CK5 positive cells are indicated. In Pg treated cells, the percentage of CK5+ cells was 21.1 times higher (95% CI: 5.2, 31.1, p<0.001) under NTC condition as compared to G2 condition, and the percentage of CK5+ cells was 12.7 times higher (95% CI: 5.2, 31.1, p<0.001) under NTC condition as compared to F11 condition. (C) Representative images of ICC stained for CK5 (red) and DAPI (blue). (D) Representative images of ICC stained for BCL6 (red) and DAPI (blue).
Figure 5. **Prolactin suppression of BCL6 and subsequently CK5 is mediated by the Stat5 pathway.** (A) Immunoblots of BCL6, CK5, phosphorylated-Stat5 (p-Stat5), Stat5a, and GAPDH protein levels in extracts of T47D cultures exposed to adenovirus carrying either Stat5a or LacZ control for 24 h and subsequently treated with vehicle (Cntrl), Prolactin (PRL), β-Estradiol (E2), or R5020 (Pg) for 48 h.  **(B)** Immunoblots of BCL6, phosphorylated-ERK1/2 (p-ERK1/2), ERK1/2, and GAPDH protein levels in extracts of T47D cultures pre-treated with Pg for 24 h followed by incubation with or without prolactin (PRL) in the presence or absence of Mek inhibitor (U0126) for 3 and 6 h.  **(C)** Immunoblots of BCL6, phosphorylated-Akt (p-Akt), Akt, and GAPDH protein levels in extracts of T47D cultures pre-treated with Pg for 24 h followed by incubation with or without prolactin (PRL) in the presence or absence of PI3K inhibitor (Ly294002) for 3 and 6 h.
Figure 6. CK5 positively correlates with BCL6 in PR-positive breast cancer tissues from premenopausal patients. (A) Representative immunofluorescence images of breast cancer tissue sections of PR-positive, premenopausal patients stained for either BCL6 (red) or CK5 (red) and cytokeratin (green) and DAPI (blue). Case 1 represents a tumor with high CK5 and high BCL6, while Case 2 represents a patient with low CK5 and low BCL6. (B) Scatter plots and correlation of levels of nuclear BCL6 and cytoplasmic CK5 proteins in PR-positive tumors of patients up to 50 years old at diagnosis (premenopausal; Spearman r=0.24, P=0.044, N=69). (C) Scatter plots and correlation of levels of nuclear BCL6 and cytoplasmic CK5 proteins in PR-positive tumors of patients older than 50 years at diagnosis (postmenopausal; Spearman r=-0.08, P=0.381, N=134).
Key Research Accomplishments

Prolactin counteracts induction of the CK5-positive population by the synthetic progestin R5020 (Pg) in luminal breast cancer cells both in vitro and in vivo.

CK5-positive cells were chemoresistant as determined by four-fold reduced rate of apoptosis following docetaxel exposure.

Pg-induction of CK5 was preceded by marked up-regulation of BCL6, an oncogene and transcriptional repressor critical for the maintenance of leukemia-initiating cells. Knockdown of BCL6 prevented induction of CK5-positive cell population by Pg. Prolactin suppressed Pg-induced BCL6 through Jak2-Stat5 but not Erk- or Akt-dependent pathways.

Clinical relevance of these findings is suggested by a positive correlation between levels of BCL6 and CK5 protein in hormone receptor-positive breast cancer among premenopausal but not postmenopausal patients, and high levels of BCL6 or CK5 correlated with unfavorable outcome.

We conclude that suppression of Pg-induced CK5-positive cells represents a novel pro-differentiation effect of prolactin in breast cancer. These insights may have direct implications for breast cancer progression and therapy since loss of prolactin receptor signaling is frequent and BCL6 inhibitors are emerging for lymphoma treatment.

Research Project 3: Project Title and Purpose

Stat5 and ErbB2 in Prostate Cancer - Organ-confined primary prostate cancer is typically treated by surgery, radiation, hormone therapy, or different combinations of these three treatment modalities, depending on the age and operability of the patient. If prostate cancer has already invaded the neighboring tissues or metastasized to distant sites by the time of the diagnosis, the main treatment options include radiation, hormone therapy and/or chemotherapy (docetaxel) combined with (neo) adjuvant therapies. However, the existing pharmacological therapies for prostate cancer only provide a temporary relief of the symptoms and the cancer growth, while the hormone-refractory form of prostate cancer develops. Moreover, no effective therapies for metastatic prostate cancer currently exist. In this project, the molecular basis of why positive activation status of transcription factor Stat5a/b may provide a biomarker for sensitivity of prostate cancer to ErbB2-inhibitors as a therapy will be examined.

Duration of Project

1/1/2009 – 06/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.
**Research Project 4: Project Title and Purpose**

*Targeting the IGF-I Receptor in Cancer* - This project proposes to determine conditions which will maximize the therapeutic effect of antibodies to the insulin growth factor I (IGF-I) receptor (a receptor for a growth factor) in cancerous tumors. Many cancers (including lung, colon and breast cancers), have already shown susceptibility to this treatment, and these antibodies are currently being used in clinical trials. Clinicians are now anxious to know which human tumors are most sensitive to the antibodies to the IGF-I receptor. There are intra- and extra-cellular factors that condition the response of tumor cells to treatment and the purpose of this project is to identify these conditions.

**Duration of Project**

1/1/2009 - 6/30/2012

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 5: Project Title and Purpose**

*The Role of MicroRNA (miRNA) Gene Expression in Therapy Resistance of Human Breast Cancer* - This year breast cancer will kill over 40,000 women in the United States, and there will be over 210,000 new cases, according to statistics from the American Cancer Society and Centers for Disease Control. Since the risk of developing breast cancer increases with age, the prevalence of this disease can be expected to increase as the overall population continues to age. However, studies on the underlying genesis and progression of breast cancer, utilizing the latest techniques of molecular biology, offer hope of finding more effective treatments. Studies over the last two years have demonstrated that the presence of “stem cells” which are found within human breast cancer, contribute to therapy resistance. This project will focus on a class of genes called the miRNA found in breast cancer stem cells, and their role in therapy resistance and breast cancer metastasis.

**Duration of Project**

1/1/2009 – 06/30/2012

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.
Research Project 6: Project Title and Purpose

Mechanisms for Metastasis Suppression through Kisspeptin Regulation of the Microenvironment

Metastasis (spreading) is an unfavorable milestone in the clinical course of cancer. The ability of tumor cells to spread from the primary site and invade normal tissues requires a specific set of properties enabling them to proliferate and migrate. It is also critical that these malignant cells have a supportive niche within which they can expand. The KiSS1 gene was discovered based on its ability to block metastasis of human cells in mouse models of melanoma. Current evidence suggests that the product of this gene is secreted by tumor cells and prevents the migrating cells from developing a niche. These experiments, which will elucidate the mechanisms of action, may provide novel, non-toxic approaches for the treatment and prevention of tumor metastasis.

Duration of Project

1/1/2009 - 12/31/2012

Project Overview

Metastasis is a grim milestone in the majority of cancers. The spread of tumor cells to secondary sites requires a cascade of properties that enable them to invade normal tissues, migrate into blood vessels, and proliferate while detached from other cells. Also required is a receptive, supportive environment that can be induced by the tumor cells through tissue remodeling, and stromal cells to provide a microenvironment that fosters growth. The cellular elements of this microenvironment include inflammatory cells, endothelial cells, fibroblasts, and myofibroblasts. The latter two populations have been designated carcinoma-associated fibroblasts, and these cells have been found to promote malignant properties. The stroma of metastatic tumors have highly similar gene expression patterns to that of wound healing, and is therefore associated with the “wound-response gene signature” of metastasis and poor prognosis.

The KiSS1 gene encodes a secreted protein that is processed to peptides (kisspeptins: KP). KP was discovered to be absent from metastatic tumors, and able to suppress metastasis in model systems. While KPs are ligands for GPR54, a G protein coupled receptor, the mechanism for metastasis suppression has not been elucidated, and the cells that express GPR54 have not been identified. Since KP expression can inhibit pulmonary metastasis from tumor cells that lack GPR54 expression, it is likely that a paracrine mechanism is active. It has been demonstrated that low levels of GPR54 are expressed by fibroblast cell lines. The objective of this project is to elucidate the paracrine mechanism responsible for metastasis suppression by kisspeptins, and to identify GPR54+ target cells responsible for these effects. The hypothesis of the project is that KP secretion decreases the formation of activated stromal fibroblasts and myofibroblasts. This hypothesis will be tested in mouse wound healing (Specific Aim 1) and pulmonary fibrosis (Specific Aim 2) models to determine whether administration of KPs or novel downsized analogs, can block the formation of the fibroblastic microenvironment.
**Expected Research Outcomes and Benefits**

Despite advances in chemotherapy, the metastatic spread of cancer is still associated with a poor prognosis, and few effective treatments are available. Kisspeptins (KPs) are ligands (bonding agents) for a G protein coupled receptor, which is the class of targets for about one-third of drugs. Thus, the demonstration that KPs are effective anti-metastatic agents will open the door to a new type of therapy. Prior to the pursuit of a therapeutic role for KPs, it is critical to develop an understanding of the precise mechanisms for suppression of metastasis, which to date has been elusive. The available data indicates that KPs do not directly inhibit the spread of tumor cells. Instead, there is evidence that they control the local environment for migrating tumor cells and prohibit the formation of a supportive niche. Thus, it is possible that a therapy based on KPs would block the ability of tumor cells to establish a receptive environment.

One approach to determining the mechanism for metastasis suppression by KPs, is to study a similar environment and identify its targets that express the corresponding receptor (GPR54). Since there is evidence that GPR54 is expressed in higher levels by lung fibroblasts, we will characterize the effects of KPs to identify relevant targets and mechanisms in pulmonary fibrosis models and in wound healing using mice. This project will also provide insight into the effects of KPs on regenerative processes while maintaining relevance to tumor therapy, because the wound healing environment has extensive molecular similarity to that of malignant tumors. The targeting of drugs to block host responses instead of tumor functions has the potential advantages of decreased toxicity and decreased emergence of resistant tumor cells.

**Summary of Research Completed**

We previously reported that activation of GPR54 inhibited Akt phosphorylation after the activation of epidermal growth factor receptor and the insulin receptor and triggered apoptosis in epithelial and lymphoid cell lines, indicating that Akt is a key signaling molecule in regulating the effect of KISS1-GPR54. Glycogen synthase kinase 3β (GSK3β) is a downstream molecule of Akt. We showed that Akt activation promoted the phosphorylation of GSK3β, which decreases GSK3β activity and results in a disassociation of the axin/β-catenin complex for β-catenin.
catenin induction and for promoting cancer metastasis. During our study using human ovarian cancer tissues, we obtained some additional interesting findings as follow:

1) Human ovarian cancer tissue had higher levels of endogenous reactive oxygen species (ROS). To study the levels of ROS generation in human ovarian cancer tissues, we collected ovarian cancer frozen tissues in tissue bank of 26 different patients with histologically diagnosed malignant ovarian cancer and 8 healthy normal ovarian tissues without evidence of any other type of cancer. Serial 10 µm frozen sections were prepared and mounted on slides coated with 3-amino propyltriethoxy silane and processed immediately. The tumor intracellular ROS levels were detected using CM2-DCFH-DA, in which CM2-DCFH-DA diffused into the cell and was hydrolyzed by intracellular esterases to polar 2',7'-dichlorofluorescin and this non-fluorescent fluorescein analogue was oxidized to highly fluorescent 2',7'-dichlorofluorescein by intracellular oxidants. The tissue sections were washed by cold 1×PBS buffer, and incubated with 10 µM CM2-DCFH-DA for 90 min. The sections were washed by cold 1×PBS and visualized under fluorescence microscope. The results showed that ovarian cancer tissues showed 10-fold higher levels of ROS when compared to normal ovarian tissues (Fig. 1).

2) ROS induced CXCL8 expression through GSK3β. To test whether ROS stimulates CXCL8 expression through GSK3β, ovarian cancer cells OVCAR-3 and A2780 were transduced with adenovirus carrying GFP or GSK3β for 24 h. After culture in serum-free medium for 24 h, cells were treated with H2O2 (100 µM). The levels of CXCL8 were tested by RT-PCR analysis. We found that H2O2 treatment induced CXCL8 expression, while overexpression of wild-type GSK3β significantly inhibited H2O2-induced CXCL8 expression (Fig. 2A). On the contrary, overexpression of dominant negative form of GSK3β (K85 mutant, K85M) enhanced CXCL8 expression in ovarian cancer cells (Fig. 2B), suggesting that ROS stimulates CXCL8 production through GSK3β.

3) The higher expression levels of p-GSK-3β and CXCL8 were associated with ovarian cancer development. CXCL8 is important for cancer metastasis. Next, we determined the expression levels of CXCL8 and phosphor-GSK3β (p-GSK3β) in ovarian cancer tissues, and analyzed the correlations between levels of CXCL8 and phosphor-GSK3β (p-GSK3β) in cancer and normal tissues. We detected the expression levels of CXCL8 and p-GSK3β proteins in cancer tissue samples by immunoblotting, and found that higher levels of both CXCL8 and p-GSK3β proteins were detected in the cancer tissues, and that the expression levels of CXCL8 and p-GSK3β were significantly correlated with ovarian cancer development (Fig. 3). These results suggest that p-GSK3β levels are correlated with the induction of CXCL8 expression in vivo, and support the important and clinical relevance of our new findings: GSK3β regulates CXCL8 expression. Since the KISS1 receptor GPR54 can inhibit Akt activation, the effect of GPR54 on ROS generation, p-GSK3β and CXCL8 expression should be tested to elucidate the mechanism of GPR54 in suppressing ovarian cancer invasion and metastasis in the future.
Fig. 1 Malignant ovarian tumor tissues showed higher levels of ROS. The levels of ROS in malignant ovarian tumor tissues and normal ovarian tissues were determined by ROS staining and quantified. The representative images (left panel), quantitative analysis of the relative levels of ROS in the normal and cancer tissues (right panel).
Fig. 2. ROS induced CXCL8 expression through GSK3β. (A) OVCAR-3 and A2780 cells were infected by adenovirus carrying GFP or GSK3β at 20 MOI for 24 h, and cultured in serum-free medium for 24 h and stimulated by H$_2$O$_2$ (100 µm) for 4 h. (A) The relative CXCL8/GAPDH mRNA levels were analyzed using total RNAs prepared from the cells. (B) Cells were infected by adenovirus carrying GFP or a dominant negative GSK3β construct (GSK3β-K85M) at 20 MOI and cultured for 24 h. Total RNAs were used to analyze CXCL8 and GAPDH mRNA levels as above. The values below the figure are the mean ± SD from 3 independent experiments. * indicates that the value is significantly different when compared to that of the control (p<0.05). # indicates the value is significantly different when compared to that of cells infected by GFP adenoviruses (p<0.05).
Fig. 3. The expression levels of p-GSK3\(\beta\) and CXCL8 were increased in human ovarian tumor tissues, and CXCL8 levels were correlated with p-GSK3\(\beta\) levels in the cancer tissues. (A) Relative CXCL8 and p-GSK3\(\beta\) protein expression levels in malignant ovarian tumor tissues and normal ovarian tissues were detected by Western blotting, and the protein signals were quantified by densitometric analysis and normalized to the signal of GAPDH. Mann-Whitney analysis assessed that CXCL8 (p=0.013) and p-GSK3\(\beta\) (p=0.0058) are differentially expressed between normal and malignant group. (B) The representative images to detect GSK3\(\beta\), p-GSK3\(\beta\), CXCL8 and GAPDH in cancer and normal ovarian tissues by Western blotting.