G31P, an Antagonist against CXC Chemokine Receptors 1 and 2, Inhibits Growth of Human Prostate Cancer Cells in Nude Mice

Xin Liu,1 Jing Peng,2 Wenchang Sun,1 Shufeng Yang,1 Guoying Deng,1 Fang Li,2* Jya-Wei Cheng3 and John R. Gordon4

1Department of Microbiology, Dalian Medical University, Dalian, P.R. China
2Department of Immunology, Dalian Medical University, Dalian, P.R. China
3Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan
4The Division of Respirology, Critical Care and Sleep Medicine, Royal University Hospital, University of Saskatchewan, Saskatoon, Canada

Prostate cancer is the most common malignancy in Western countries. Chemokine C-X-C motif receptor 1 (CXCR1) and CXCR2 play a key role in generation and regulation of CXC chemokine signaling. CXCR1 is a receptor for interleukin 8 (IL8), a pro-inflammatory chemokine, and CXCR1/2 are crucially involved in the prostate cancer development and progression. Thus, we generated a high-affinity human CXCR1/CXCR2 inhibitor, CXCL8 (3-72) K11R/G31P, named G31P, which is a synthetic derivative of the human cytokine, IL-8. In this study, we investigated the effects of G31P on regulation of prostate cancer cell growth in vitro and in nude mouse xenografts. Cell viability, adhesion, and wound healing assays were used to assess the effects of G31P on growth, adhesion, and migration of PC-3 human prostate cancer cells in vitro, respectively. Nude mouse xenografts and xenograft implantation assays were performed to determine the effect of G31P on PC-3 cells in vivo. Immunohistochemistry was used to detect gene expression, and fluorescence imaging was used to detect tumor volume and microvessel density in tumor xenografts. The data showed that G31P treatment significantly reduced PC-3 cell viability, adhesion and migration capacity in a dose-dependent manner (up to 100 ng/ml). Additionally, G31P treatment of nude mice suppressed the growth of orthotopically transplanted tumor xenografts. G31P also inhibited tumor tissue vascularization, which was associated with the decreased expression of vascular endothelial growth factor and nuclear transcription factor (NF)-κB in orthotopic xenograft tissues. This study provides evidence that G31P, a CXCR1/2 inhibitor, may effectively control prostate cancer.

Keywords: chemokine; CXCR1; CXCR2; G31P; prostate cancer

Received April 13, 2012; accepted September 6, 2012. Published online September 26, 2012; doi: 10.1620/tjem.228.147. Correspondence: Fang Li, Department of Immunology, Dalian Medical University, 9 Western Lushun South Road, Dalian, P.R. China. e-mail: lifang16@hotmail.com

Prostate cancer is the most common malignancy in the Western countries and the second leading cause of cancer-related deaths in American men (Jemal et al. 2005, 2007). In early stages, most prostate cancers are slowly growing and approximately 30% progress to advanced stages with extensively invasive and metastatic diseases. Unlike most other cancers, treatment of prostate cancer depends on the grades and stages of the disease. For example, a low-grade (Gleason score 6 or below) tumor, if occurring in elderly men and growing slowly, may not need any treatment at all, but just requires active surveillance (http://www.netdoctor.co.uk/diseases/facts/prostatecancer.htm). However, for aggressive prostate cancer, treatments include curative surgery, radiation therapy, hormonal therapy, chemotherapy or their combination, and the standard treatment of advanced prostate cancer is androgen ablation (Nakabayashi et al. 2011; Nishiyama 2012). The latter treatment induces apoptosis of androgen dependent tumor cells. Nevertheless, long-term treatment will induce tumor cells to be androgen-independent, thus tumor cells will survive, proliferate, and disseminate. Moreover, androgen-independent prostate cancer is largely refractory to other types of chemotherapy regimens (Wilson et al. 2008). These patients usually die within a few years after the tumor becomes refractory (Mohapatra et al. 2009). Therefore, it is urgent to develop novel approaches to treat aggressive, but refractory prostate cancers, to prolong patient survival and increase quality of life.

The CXC chemokine subfamilies are characterized by the presence of two cysteine residues at their amino termini (Bizzarri et al. 2006). Among them, CXCL8 (interleukin-8) is produced by macrophages and epithelial cells or endothelial cells and plays a central role in promoting neutrophil recruitment to inflammatory sites and inflammatory response (John et al. 2005). It is also a potent angiogenic factor, and more recently, it was found to possess tumori-
genic properties (Reiland et al. 1999; Lee et al. 2004; Araki et al. 2007; Maxwell et al. 2007; MacManus et al. 2007; Waugh and Wilson 2008). In prostate cancer, this multifunctional chemokine is thought to be involved in cancer development and progression and resistance to chemotherapy (Araki et al. 2007; Luppi et al. 2007). Previous studies demonstrated that CXCL8 treatment stimulated proliferation of prostate cancer cells, and CXCL8 overexpression was associated with tumorigenicity, lymph node metastasis and angiogenesis of androgen-independent prostate cancer in athymic nude mice (Inoue et al. 2000; Kim et al. 2001). Furthermore, CXCL8 was elevated in the sera of prostate cancer patients, and the chemotherapy induced CXCL8 expression, which resulted in the reduced sensitivity of prostate cancer cells to apoptosis (Wilson et al. 2008).

Molecularly, CXCL8 binds to the seven-transmembrane G-protein coupled receptors, CXC chemokine receptor (CXCR) 1 and CXCR2 (or CXCL8R2), with different expression and affinity to different chemokines (Cerretti et al. 1993). For example, CXCL6 (granulocyte chemoattractant protein-2; GCP-2) and CXCL8 can bind to CXCR1 with high affinity, while all of the Glu-Leu-Arg (ELR)-CXC chemokines can bind to CXCR2 with lower affinity (Holmes et al. 1991; Murphy and Tiffany 1991). CXCL8 and the two CXCL8 receptors are expressed in prostate cancer tissues (Murphy et al. 2005). Furthermore, among the ELR-CXC chemokines, CXCL1-3 [growth-related oncogene (GRO)-α, -β, and -γ] are highly expressed in colon cancer cells (Doll et al. 2010) and also in tumor-infiltrating neutrophils, tumor-associated macrophages, and endothelial cells, suggesting that multiple ELR-CXC chemokines can regulate the tumor microenvironment (Brat et al. 2005; Charalambous et al. 2005). Clinical studies have confirmed the increased expression of CXCL8 in the more advanced stages of cancers, which indicates that suppression of ELR-CXC chemokine activity may be an important therapeutic approach for aggressive and metastatic tumors (Waugh and Wilson 2008).

Therefore, our research aimed to target ELR-CXC chemokines to effectively control different human cancers. In our previous study, we produced a broad-spectrum ELR-CXC chemokine inhibitor, CXCL8 (3-72) K11R/G31P (G31P) (Li et al. 2002a, 2002b; Zhao et al. 2009), and showed its promising anti-tumor activities in multiple model systems (Zhao et al. 2010). G31P contains arginine instead of lysine at position 11 of CXCL8 and proline instead of glycine at position 31 of CXCL8. In this study, we investigated the effects of G31P on the proliferation, adhesion and migration of the androgen-independent prostate cancer cells in vitro. Additionally, we examined the effects of G31P on growth and angiogenesis in nude mouse xenografts of prostate cancer.

Materials and Methods

Cell line and culture

Human prostate cancer PC-3 cells were obtained from the American Type Culture Collection (Manassas, Virginia, USA) and maintained in a complete RPMI 1640 medium containing 10% fetal bovine serum (FBS), with 1% Ampicillin and streptomycin (all from Hyclone, Australia) at 37°C in humidified 5% CO₂ and 95% air atmosphere.

Cell viability assay

Cell viability was determined using a commercial kit (WST-8 Cell Counting Kit-8, Sigma Chemical Co, St Louis, MO, USA), as described in a previous study (Shoji et al. 2009). PC-3 cells were trypsinized with 0.05% trypsin after achieving 80% confluency and then seeded into 96-well plates (5 × 10⁴ cells/well) in a complete RPMI 1640 medium. Twenty-four hours later, the cells were treated with or without G31P at a dose of up to 100 ng/ml for 72 h at 37°C. At the end of the experiments, 10 μl of the cell counting kit reagent was added into each well and the plates were incubated for an additional 4 h. The absorbance rate at 450 nm was determined using a 96-well plate reader. The experiment was in triplicate and repeated at least twice.

Cell adhesion assay

To detect tumor cell adhesion properties, we pre-coated 96-well plates with cell adhesion reagents (extracellular matrix, ECM, 10 μg/ml; Sigma) and then rehydrated them with phosphate buffered saline (PBS). Next, the wells were filled with 1% heat-denatured bovine serum albumin (BSA) for 1 h at 37°C in a humidified atmosphere of 5% CO₂ and then washed three times with PBS. PC-3 cells were grown and treated with or without G31P (up to 100 ng/ml) in a serum-free medium for 24 h. Then, the cells were seeded at 3 × 10⁴ cells/well in the pre-coated plates, incubated for 1 h at 37°C, and gently washed three times with PBS. The remaining adhered cells were enumerated using the same cell counting reagent as above, and the absorbance rate at 450 nm was determined with a 96-well plate reader (Takami et al. 2002).

Cell wound healing assay

To detect tumor cell migration capacity, we performed a wound-healing assay as described previously (Zhang et al. 2010). Briefly, the tumor cells were cultured in 12-well plates under standard culture conditions and allowed to reach 100% confluence. Next, a ‘wound’ was created in the confluent monolayer with a 200-μl-pipette tip and then the wells were rinsed three times with PBS. The cells were incubated in the complete medium at 37°C for up to 72 h in the presence of 0-100 ng/ml G31P. The migration rate of the PC-3 cells at the indicated period of time points was evaluated using an inverted microscope. The wound-healing rate was quantified as the distance the cells migrated across the injury line during the culture. A total of three ‘wounds’ per well were used to generate the data, and the experiments were completed in duplicate and repeated at least once.

GFP vector and gene transfection

A pEGFP plasmid containing an enhanced green fluorescent protein (EGFP) tag was obtained from Takara Biotechnology (Dalian, China) for gene transfection to visualize prostate cancer cells in nude mice. This vector contained the human cytomegalovirus immediate
early promoter to drive expression of green fluorescent protein in cells. For gene transfection, PC-3 cells were seeded and grown for 72 h to reach 40% confluence with a 1:1 mixture of precipitated retroviral RT67 (a mouse retrovirus packaging cell line) cell supernatants and RPMI 1640 medium containing 10% FBS. Next, the culture medium was replaced with the complete medium and the cells were harvested using 0.25% trypsin and sub-cultured at 1:15 using the gene selective medium containing 200 μg/mL G418 (the geneticin® selective antibiotic). The level of G418 was increased to 1,000 μg/mL in a stepwise manner in order to select brightly fluorescent cells. After, the fluorescent PC-3 cells were passaged and expanded using conventional culture methods in the absence of the selective agent.

Nude mouse xenograft assay

Athymic male nude mice (4-6 week-old BALB/c) were obtained from the Yuanduan Lab Service Corporation (Nanjing, China) and maintained in a laminar airflow cabinet under the specific pathogen-free conditions. This study was approved by our Institutional Animal Care and Use Committee of Dalian Medical University. The animals freely accessed to tap water and standard pellet food, and their health was monitored daily. For the nude mouse xenograft assay, the monolayer-cultured GFP-positive PC-3 cells (PC-3-GFP) were harvested and inoculated subcutaneously into the right flank of three nude mice with 5 × 10⁶ cells per mouse. The tumors were allowed to grow for 2 to 4 weeks, and then were harvested for implantation when palpable. At the end of the experiments, the tumor xenografts from these three mice were resected, sliced into 1-mm³ fragments, and then implanted to the prostate tissues of the recipient nude mice under local anesthesia and sterile surgical conditions. A total of 24 animals received tumor fragment implants. Five days later (day 0), the animals were assigned into two groups (12 animals/group) for subcutaneous injection of normal saline (N.S.) or G31P (0.5 mg/kg) at every other day for 24 days. GFP fluorescence images of the growing tumors were captured on days 12, 18 and 24 using a digital camera under the optical configuration of a dissection microscope with a 515 nm emission filter. The tumor volumes were calculated using a formula: Volume = (length × width²)/2. Length and width measurements were determined with Image-Pro 6.0 Microsoft (Media Cybernetics, Bethesda, MD, USA). On day 24, all the mice were sacrificed and GFP fluorescence images of the tumors were captured. Vascular microvessel density was calculated using the formula: Density = microvessel length/tumor area. Tumor samples from each mouse were fixed in 4% paraformaldehyde and embedded in paraffin using standard procedures for subsequent immunohistochemical analyses.

Immunohistochemistry

Paraffin-embedded prostate cancer xenograft sections were dewaxed and rehydrated in PBS using standard procedures. The sections were rinsed three times with PBS and heat-treated for 15 min in 10 mM sodium citrate (pH 6.0). After, the endogenous peroxidase activities were blocked by treatment with 3% hydrogen peroxide for 10 min, the sections were rinsed three times with PBS, incubated with a protein-blocking solution of 5% normal horse serum in PBS (pH 7.5) for 15 min at room temperature, washed three times with PBS, and then incubated with a mouse monoclonal anti-VEGF (vascular endothelial growth factor) antibody (1:50), a rabbit polyclonal anti-NF-κB (nuclear transcription factor-κB) p65 antibody (1:50), or a goat polyclonal anti-CD31 (platelet endothelial cell adhesion molecule-1) antibody (1:50) for 20 h at 4°C. CD31 was used as a endothelial cell marker. In the next day, the sections were washed with PBS three times and incubated with the appropriate dilution of the secondary antibody (anti-mouse IgG, anti-rabbit IgG or polymer helper) for 40 min at 37°C. After washing three times with PBS, the sections were incubated with biotinylated goat anti-mouse, or anti-rabbit-poly peroxidase anti-goat immunoglobulin for 30 min in the dark. For color development, the sections were washed with PBS three times, incubated in diaminobenzidine solution for 10 min, and then counterstained with hematoxylin for 1 min (Kollmar et al. 2007). The negative control sections were incubated with PBS instead of the primary antibody. The intensity of the stained sections were measured after converting photographed sections into gray scale (scale 0-225) and represented by integrated optical density (IOD), which was calculated using Image-Pro 6.0 Microsoft.

Statistical analyses

All the data are summarized as the mean ± standard deviation (s.d.), when it was appropriate. The differences between the experimental and control groups were calculated using one-way analysis of variance (ANOVA) followed by the appropriate post-hoc comparison. All statistical analyses were performed using SPSS 10.0 for Windows software (SPSS, Chicago, IL, USA). Statistically significant differences were accepted when p ≤ 0.05.

Results

Effects of G31P on regulation of prostate cancer cell viability, adhesion, and migration

We first assessed the effects of G31P on regulation of prostate cancer cell viability and adhesion capacity in vitro. We found that G31P reduced prostate cancer cell viability in a dose-dependent manner (Fig. 1A). At a dose of 100 ng/ml G31P, tumor cell viability reduced by 40% compared to the control cells (p < 0.01). Moreover, tumor cell adhesion to ECM reduced 35% after treating cells with 1 ng/ml G31P (Fig. 1B). Then, we determined the effects of G31P on regulation of prostate cancer migration using a wound-healing assay. Our data showed that G31P, at a dose of 10 or 100 ng/ml, significantly reduced the ability of PC-3 cells to migrate, as determined at either 48 h or 72 h when compared to the control cells (p ≤ 0.05; Fig. 1C).

Effect of G31P on suppression of prostate cancer xenograft growth in vivo

Next, we determined the effects of G31P on regulation of nude mouse xenograft formation and growth. We first transfected a GFP expression vector into PC-3 cells and then injected these cells into nude mice and treated them with G31P (0.5 mg/kg) for up to 24 days. The drug dose chosen for the in vivo study was based on our previous in vivo study (our unpublished data). Fluorescent images of
Fig. 1. Effects of G31P on the inhibition of prostate cancer PC-3 cell viability, adhesion, and migration.

A. Cell viability assay. PC-3 cells (5 x 10^3 cells/well) were treated with or without G31P at a dose of up to 100 ng/ml for 72 h. B. Cell adhesion assay. PC-3 cells were pretreated with G31P at a dose of up to 100 ng/ml for 24 h. C. Cell wound healing assay. PC-3 cells were cultured, allowed to grow to 100% confluence, and then subjected to ‘wounding’. The cells were then incubated with G31P at various concentrations (0, 0.1, 1, 10, 100 ng/ml) in RPMI 1640 medium containing 1% FBS for 0, 24, 48 or 72 h at 37°C (5% CO2). Migration of the PC-3 Cell was observed by microscopy at the indicated time points (x 400). The bottom graph summarized the relative migration rate of PC-3 cells. The wound-healing rate was quantified as the distance the cells migrated across the injury line during the culture. The graphs represent the mean ± s.d. values (n = 3) of triplicate cultures. *p < 0.05 vs. control.
Fig. 2. Effect of G31P on the suppression of prostate cancer xenograft growth in nude mice.

A and B. Fluorescence images of tumor xenografts in the N.S control mice (A) and G31P-treated mice (B), respectively, on day 24 (abdominal cavity opened). The images were captured on day 24. C. Fluorescence images in the N.S control and G31P-treated nude mice on day 12, 18 and 24 (*p < 0.05, **p < 0.01 vs. N.S control). D. Summary data of tumor volumes. E. Summary data of tumor weight on day 24. The graphs represent the mean ± s.d. of xenografts from 12 animals.
the GFP-expressed tumor xenografts in mice captured on day 12, 18 and 24 demonstrated that G31P treatment significantly reduced prostate cancer xenograft formation and growth (Fig. 2A-D). On day 24, the tumor xenograft volume was 25% of the control (Fig. 2D; p < 0.01). The weight of the xenografts was also significantly reduced as compared to the controls (Fig. 2E; p < 0.05).

**Effect of G31P on inhibition of angiogenesis in tumor xenografts**

We measured angiogenesis of the tumor xenografts after the mice were sacrificed on day 24. GFP fluorescence images were captured using a digital camera mounted on a dissecting microscope (Fig. 3A) and the microvessel density of each tumor was calculated using Image-Pro 6.0 software. The data showed that G31P treatment significantly reduced formation of tumor blood vessels in the treatment group when compared to the saline control group (Fig. 3B; p ≤ 0.05). Representative CD31-stained microscopic photos are shown in Fig. 4. The MVD was significantly lower in the G31P-treated xenografts (0.124% ± 0.038) than that of the control xenografts (0.721% ± 0.223; p ≤ 0.05). Molecularly, the expression of VEGF protein, an important mediator in angiogenesis, and NF-κB, an inflammation response modulator and angiogenesis factor, were decreased in tumor xenografts after G31P treatment compared to that of the control mice (Fig. 5). In particular, the intensity of VEGF (1.7 × 10^4 vs. 5.7 × 10^4, p < 0.01) and NF-κB (1.0 × 10^4 vs. 2.7 × 10^4, p < 0.01) staining, as represented by IOD (integrated optical density) of tumor xenografts treated with G31P was significantly reduced compared to that of the control xenografts at day 24 (Fig. 5).

**Discussion**

G31P is a low molecular weight ELR-CXC chemokine inhibitor that has a higher binding affinity for CXCR1/2 than any other chemokine receptor (Zhao et al. 2009). In this study, we investigated the effects of G31P on the regulation of cell viability, adhesion and migration of androgen-independent prostate cancer PC-3 cells in vitro. Additionally, we investigated tumor xenograft formation and growth in nude mice. Our data demonstrated that G31P reduced prostate cancer cell viability and inhibited their adhesion and migration capacity in a dose-dependent manner in vitro. Furthermore, G31P treatment of nude mice inhibited formation and growth of prostate cancer cells in orthotopically-transplanted xenografts. As expected, G31P treatment also reduced xenograft microvessel formation and the expression of VEGF and NF-κB proteins in xenograft tissues. Our present study provides a novel therapeutic approach in future clinical control of aggressive prostate cancer.

Indeed, increased expression of ELR-CXC chemokines and their receptors has been observed in many different cancer cells, including tumor-infiltrating neutrophils, tumor-associated macrophages and endothelial cells (Brat et al. 2005; Charalambous et al. 2005). ELR-CXC chemokine expression could play an important role in tumor microenvironment modifications (Brat et al. 2005; Charalambous et al. 2005). Moreover, a previous study showed increased expression of CXCL8 in advanced cancers, which could provide an important therapeutic approach for aggressive and metastatic tumors (Waugh and Wilson 2008). Another study demonstrated therapeutic effects of anti-CXCL8 antibodies in animal models of cancer (Bao et al. 2010). Since many ELR-CXC chemokines play roles in human cancers, neutralization of only CXCL8 may not be sufficient and effective. Thus, multiple small molecule inhibitors of the most important chemokine receptors, CXCR1 and CXCR2, have been generated. A few of these inhibitors showed efficacy in the treatment of experimental cancers (Bolitio et al. 2010; Tazzyman et al. 2011; Varney et al. 2011). In this study, we determined the effects of our newly synthesized CXCR1/2 inhibitor G31P in prostate cancer cells. We found that treatment with G31P, at concentrations between 10 ng/ml and 100 ng/ml significantly reduced the viability of prostate cancer cells. G31P also inhibited tumor cell adhesion and migration abilities. In nude mice, G31P suppressed growth of orthotopically-transplanted tumor xenografts and inhibited tumor vascularization and angiogenesis factors, such as VEGF and NF-κB. These anti-tumor effects of G31P were similar to other drugs, such as Taxol (our unpublished data). A G31P concentration of 0.5 mg/kg is the effective dose, but easily achievable in the clinic; however, the toxicity of G31P should be investigated in future in vivo studies.

The attainment of tumor cell invasiveness and migration is an important aspect of tumor progression. Chemokines, including CXCL8, can modulate adhesion, invasion and migration of a variety of tumor cells, such as prostate cancer cells (Li et al. 2005; Gabellini et al. 2009; Singh et al. 2011). Our current study further confirmed these data. In addition, CXCL8 is a potent mediator of angiogenesis. In this study, we demonstrated that the microvessel density of tumor xenografts was significantly suppressed by G31P treatment when compared with the saline control group. This further illustrated the effects of G31P in the suppression of cancer progression.

Numerous in vitro and in vivo studies have suggested that NF-κB plays an important role in regulating cell proliferation, adhesion, invasion, angiogenesis, and metastasis (Hinz et al. 1999; Patel et al. 2004; Voboril et al. 2004). In addition, VEGF is a NF-κB-inducible protein that is the most specific and potent angiogenic factor in tumor-induced angiogenesis. A number of studies have demonstrated that the metastatic potential of tumor cells is associated with VEGF overexpression through activation of the NF-κB pathway (Stillie et al. 2009). In the current study, we demonstrated that G31P treatment suppressed the expression of both VEGF and NF-κB in tumor xenograft tissues. Thus, the inhibition of VEGF expression via the NF-κB pathway may be a potential mechanism by which G31P inhibits
CXCR1/2 Antagonist in Prostate Cancer

Based on the current study, we speculate that G31P could be effective as an anti-cancer agent in a variety of cancerous types. However, further in-depth investigations of the dose-dependent responses and toxicity of G31P will provide useful and insightful data to support our hypothesis.

prostate cancer cells (Shishodia and Aggarwal 2004; Zhu et al. 2010).

Fig. 3. G31P inhibition of prostate cancer xenograft angiogenesis in nude mice.
Xenografts for GFP-transfected prostate cancer PC-3 cells were implanted into the prostate of nude mice and treated with or without G31P for 24 days. On day 24, all the mice were sacrificed and GFP fluorescence images of the tumors were captured. Vascular microvessel density was calculated using the formula: Density = microvessel length/tumor area. The bottom graph shows the summarized image data with mean ± s.d. values from 12 mice. *p < 0.05 vs. N.S control.

Fig. 4. Effect of G31P on the regulation of CD31 expression in prostate cancer cell mouse xenografts.
Tissue sections from the control (saline-treated) and G31P-treated xenografts were immunostained for CD31 and then the data were quantified, compared, and summarized as low and high microvessel density (MVD). The MVD was significantly lower in the G31P-treated xenografts (0.124% ± 0.038) than that of the control xenografts (0.721% ± 0.223; p ≤ 0.05). The data show that tumor sections from the control tissues had high MVD, whereas tumor sections from G31P-treated xenograft tissues had low MVD (magnification, × 400). Arrows, microvessels.
Fig. 5. Effect of G31P on regulation of VEGF and NF-κB expression in prostate cancer cell mouse xenografts.  

A. Immunohistochemical analysis of VEGF expression in the control and G31P-treated mouse xenograft tissues (magnification, ×400). The bottom graph shows the summarized data of VEGF staining as mean ± s.d. values. **p < 0.01 vs. control. Arrows, positive VEGF expression.  

B. Immunohistochemical analysis of NF-κB expression in the control and G31P-treated mouse xenograft tissues (magnification, ×400). The bottom graph shows the summarized data of NF-κB staining as mean ± s.d. values. **p < 0.01 vs. control. Arrows, positive NF-κB expression.
Acknowledgments

This work was supported in part by a grant from the National Science Foundation of China (NSFC30772023) and from the Natural Sciences and Engineering Research Council of Canada (JRG).

The Authors’ Contribution

Xin Liu performed nude mouse xenograft and cell assays, wrote the manuscript, and helped design the study; Jing Peng and Wenchang Sun performed immunohistochemistry; Shufeng Yang and Guoying Deng performed cell culture and various assays; Jya-Wei Cheng constructed the GFP vector and gene transfection; Fang Li and John R. Gordon prepared G31P and helped design the study.

Conflict of Interest

The authors declare no conflict of interest.

References


