A novel antitumor activity of deguelin targeting the insulin-like growth factor (IGF) receptor pathway via up-regulation of IGF-binding protein-3 expression in breast cancer

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Abstract

In this study, we investigated the antitumor effects of deguelin in several human breast cancer cells in vitro and in vivo. Deguelin inhibited cell viability and the anchorage-dependent and anchorage-independent colony formation of triple-negative (MDA-MB-231 and MDA-MB-468) and triple-positive (MCF-7) breast cancer cells, and it significantly reduced the growth of MCF-7 cell xenograft tumors. The induction of apoptosis, inhibition of insulin-like growth factor-1 receptor (IGF-1R) signaling activation, and up-regulation of IGF-binding protein-3 (IGFBP-3) expression may be associated with deguelin-mediated antitumor effects. Our findings suggest a potential therapeutic use for deguelin in patients with triple-negative breast cancer and for those with breast cancers who are sensitive to endocrine- and HER2-targeted therapies.

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1. Introduction

Breast cancer is the most frequent type of cancer for women worldwide. Approximately 10% of women in the United States develop breast cancer. Endocrine therapy is the choice for the treatment of patients with hormone-sensitive breast cancer. However, tumors develop the ability to escape the antiproliferative effects of endocrine therapy, and many patients become resistant to this therapy [1]. The mechanisms of endocrine resistance involve estrogen hypersensitivity, the up-regulation of alternative signal transduction pathways, and crosstalk between up-regulated signal transduction pathways and the estrogen receptor (ER) pathway [2,3]. Therefore, the development of new therapeutic strategies capable of regulating the biological pathways involved in cancer formation and progression that are independent of hormone receptor status may greatly improve the treatment of patients with breast cancer.

Deguelin, a natural product isolated from several plant species, including Mundulea sericea, has shown great potential as a cancer chemopreventive and therapeutic agent for a number of cancers, including those of the lung and breast [4–6]. The deguelin antitumor effects have been associated with cell cycle arrest and apoptosis through multiple mechanisms, mainly including the inhibition of the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK)-mediated signaling pathways [4]. Recent studies have demonstrated that deguelin is capable of suppressing Hsp90 function by binding to the Hsp90 ATP-binding pocket, leading to the destabilization of a variety of client Hsp90 proteins, including Akt, CDK4, hypoxia-inducible factor 1α (HIF-1α), and MEK [6], and thus the suppression of tumor growth [7]. Consistent with these findings, deguelin has demonstrated the capacity to circumvent radio-resistance by decreasing radiation-induced Akt signaling and HIF-1 expression in breast cancer and non-small cell lung cancer (NSCLC) cells, respectively [7,8]. Studies have also revealed that deguelin induces a rapid depletion of ATP levels and the activation of AMP-activated protein kinase (AMPK), and it inhibits the mammalian target of rapamycin-mediated increase in the expression of the survivin protein, an inhibitor of apoptosis protein (IAP), by regulating AMPK and Akt activities [9]. Survivin overexpression has been observed in most common tumor types, including more than 70% of human breast tissues. Recent studies have demonstrated that survivin is a reliable marker for aggressive disease, resistance to chemo- or radio-therapy, and poor human cancer prognosis [10]. Therefore, the suppression of
survivin expression may be another mechanism of deguelin-med-

This study was conducted to evaluate the effects of deguelin on
triple-negative breast cancer cells and the growth of breast cancer
cell xenograft tumors and to explore potential biomolecules in-
volved in the antitumor activities of deguelin in breast cancer cells.
We demonstrated that deguelin exerts its antitumor activities in
ER-, PR-, and HER2-positive breast cancer cells as well as triple-
negative cells at least in part by up-regulating IGF binding pro-
tein-3 (IGFBP-3) and inhibiting the expression of IGF-IR signaling
components. These results suggest that deguelin is an effective
therapeutic agent targeting the IGF-1R pathway for the treatment of
breast cancer.

2. Materials and methods

2.1. Human cancer cell lines, chemicals, and reagents

The deguelin (97–98% purity, HPLC-UV) used in this study was synthesized
from commercially available rotenone, as previously described [12]. Deguelin was
dissolved in dimethyl sulfoxide (DMSO) at a 10−4 M stock concentration and stored
at −80 °C. The mammalian IGF-1R expression vector was kindly provided by Dr. Lee
M. Ellis (UT MD Anderson Cancer Center, Houston, TX, USA). Control and IGFBP-3
shRNA plasmids were purchased from Sigma–Aldrich (St. Louis, MO, USA). The hu-
man breast cancer cell lines MCF-7, MDA-MB-468, and MDA-MB-231 were ob-
tained from the American Type Culture Collection (Manassas, VA). Cells were main-
tained in RPMI 1640 or DMEM/F12 (50:50) (Mediatech, Herndon, VA, USA)
supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), 2 mM l-glut-
tamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin (Mediatech) in a humid-
ified environment containing 5% CO2.

2.2. Cell proliferation assay

Breast cancer cells were treated with different concentrations of deguelin in
DMEM supplemented with 10% FBS. Cell proliferation was measured using the 3-(4,5-
dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

2.3. Clonogenic growth assay

The anchorage-dependent clonogenic growth assay was performed by seeding
breast cancer cell lines into 6 well plates at a low density (400–500 cells/well). Cells
were treated with different deguelin concentrations (0.01, 0.1, and 1 µM) in DMEM
supplemented with 10% FBS for 7–10 days in a humidified atmosphere with 5% CO2
at 37 °C. The colonies were stained with 0.1% crystal violet (Bio-Rad Laboratories,
Hercules, CA, USA) in 30% methanol and 10% acetic acid or hematoxylin. We then
counted the number of colonies with more than 50 cells.

2.4. Soft agar colony formation assay

To test the effect of deguelin on anchorage-independent colony formation, MCF-
7, MDA-MB-231, and MDA-MB-468 tumor cells were suspended in 0.4% top agar
dissolved in growth medium at 1 × 10^4 cells/ml and then placed on top of solidified
base agar (1%) in 24-well plates. After the top cell layer solidified, growth medium
containing DMSO or deguelin was overlaid. Two weeks later, colonies greater than
125 µm in diameter were counted using an inverted microscope at 40× magnifica-
tion after staining with an MTT solution.

2.5. Cell-cycle and apoptosis assays

For cell-cycle and apoptosis assays, both the adherent and nonadherent cells
were harvested, pooled, and fixed with 90% methanol. For cell-cycle analysis, we
for each sample. Apoptosis was analyzed by double-staining with
Annexin V-FITC and PI using an Annexin V-FITC apoptosis detection kit (BD Bio-
sciences), according to the manufacturer's recommended procedure.

2.6. Western blot analysis

Total protein was isolated and subjected to Western blot analysis as previously
described [11]. The following antibodies were used for the Western blots: rabbit
polyclonal antibodies detecting survivin and IGFBP-3 (Santa Cruz Biotechnology,
Santa Cruz, CA, USA); pAkt (S473), pERK, and Akt (Santa Cruz); IGFBP-3 (Diagnostic Sys-
tems Laboratory (DSL), Webster, TX, USA); cleaved poly(ADP-ribose) polymerase
(PARP) (BD Biosciences); pIGF/IR (Y1162/63) (Invitrogen); and β-actin (Sigma–Al-
drich). After incubation with corresponding secondary antibodies for 1 h, signals
were detected using an enhanced chemiluminescence (ECL) kit (Thermo Fisher Sci-
etific, Inc., Rockford, IL, USA).

2.7. Mouse xenograft breast cancer models

All animal procedures were performed in accordance with a protocol approved
by the Institutional Animal Care and Usage Committee. Six-week-old female athy-
imic (nu/nu) mice with body weights of approximately 20 g were purchased from
Harlan-Sprague Dawley (Indianapolis, IN, USA) and acclimated in the laboratory
for 1 week before experimentation. To support the growth of the estrogen-depen-
dent MCF-7 cell tumors in mice, slow-release 17β-estradiol pellets (0.72 mg, 60-day
release; Innovative Research of America, Sarasota, FL, USA) were subcutane-
ously implanted into the shoulder area using a 10-gauge trocar needle 3 days before
tumor cell injection. MCF-7 cells grown in culture (5 × 10^6 cells) were suspended
in Matrigel (BD Biosciences) and transplanted by subcutaneous injection into the left
and right flanks of nude mice. To establish MDA-MB-231 cell xenograft tumors, cul-
tured MDA-MB-231 cells (5 × 10^6 cells/mouse) were subcutaneously injected into
the right flanks of nude mice. When the xenograft tumors reached approximately
75 mm³, the mice were randomly assigned to one of two groups of five animals, and
deugelin (4 mg/kg) was suspended in cottonseed oil and administered twice a
day by oral gavage to the tumor-bearing nude mice. Tumors were measured for
45 days, and tumor volumes were calculated using the following formula for an
ellipsoid sphere: W1 × W2/2 = x mm³, where W1 represents the largest tu-
or diameter and W2 is the smallest tumor diameter. Mice demonstrating necrotic
tumors or tumors ≥ 1.5 cm in diameter were euthanized. The results were ex-
pressed as the mean (±standard deviation) tumor volume (calculated from five
mice).

2.8. Immunohistochemical analysis

Tumor tissues collected from animals in each treatment group were fixed with 10% formaldehyde, embedded in paraffin, and sectioned into 5-μm-thick slices. Immunohistochemical analyses were performed as previously described. Briefly, sections were deparaffinized and immersed in methanol containing 0.3% hydrogen peroxide to block endogenous peroxidase activity. The tissue sections were incor-
bated with 2.5% blocking serum followed by incubation with a primary antibody di-
rected against pAkt (diluted at 1:200), survivin (diluted at 1:400), pIGF-1R (diluted
at 1:50), or IGFBP-3 (diluted at 1:50) overnight at 4 °C. The sections were then
tained using a standard avidin–biotin immunohistochemical technique, according
to the manufacturer’s recommendations (Vector Laboratories, Burlingame, CA,
USA). Diaminobenzidine was used as a chromogen, and commercial hematoxylin
was used as a counterstain. The results reported here represent the findings of tu-
ors from three mice in each treatment group.

2.9. Reverse transcription-PCR

To detect changes in the IGFBP-3 mRNA level after deguelin treatment, we iso-
lated cDNA using an RT-PCR kit (Invitrogen, Carlsbad, CA, USA). The primer
sequences were as follows: 5′-GGG GGA CAG CAC TCC TTG TTC-3′ (sense) and 5′-
CCA GCT CCA GAA AAT GCT AG-3′ (antisense) for IGFBP-3 and 5′-GCT CAA CGG
CCG TGT CAA CGG ATT T3-3′ (sense) and 5′-AAT GCC AAA GTC ATG CAC C-3′
(antisense) for GAPDH.

2.10. Transfection

Transfection with mammalian expression vectors or shRNAs was performed with
Lipofectamine 2000 (Invitrogen), according to the manufacturer’s recommendations.

2.11. Statistics

Data are presented as the mean ± SD. Figure data are shown as one representative
of at least two independent experiments. The statistical analysis of data acquired
in vitro experiments was performed using Student’s t-test with the Microsoft Ex-
cel software (version 14; Microsoft Corporation, Seattle, WA, USA). Statistical signif-
icance for tumor growth differences was analyzed by ANOVA. For all statistical
analyses, two-sided P values < 0.05 were considered statistically significant.

3. Results

3.1. The effects of deguelin on the growth of triple-negative breast cancer cells

We performed the MTT assay to investigate the effects of deguelin on triple-positive MCF-7 and triple-negative MDA-MB-231

breast cancer cells. After 3 days of treatment, deguelin decreased the viability of the MCF-7 and MDA-MB-231 cells in a dose-dependent manner at concentrations ranging from $10^{-8}$ M to $10^{-6}$ M (Fig. 1A). The ER-positive T47D and triple-negative MDA-MB-468 breast cancer cells also demonstrated a significant decrease in viability in response to deguelin treatment (Supplementary Fig. 1). Consistent with the MTT assay results, deguelin treatment also affected the anchorage-dependent colony-forming ability of these cells (Fig. 1B). Because the behavior, growth, and internal and external signal response of cells grown in vivo in three-dimensional conditions are largely different from those of cells grown in vitro in a monolayer on tissue culture plates [4], we tested the effects of deguelin on breast cancer cells grown in soft agar, a 3D culture system. Deguelin significantly reduced the colony forming ability of all of the breast cancer cell lines in a dose-dependent manner (Fig. 1C). MDA-MB-468 cells also demonstrated a significant decrease in the anchorage-independent colony-formation ability in response to deguelin treatment (Supplementary Fig. 2). Deguelin treatment in soft agar at concentrations higher than 100 nM caused a more than 50% inhibition in the colony forming ability of the MCF-7, MDA-MB-231, and MDA-MB-468 cells. These in vitro findings suggest that deguelin is capable of suppressing the tumorigenicity of breast cancer cells, including those with a triple-negative phenotype.

3.2. The induction of apoptosis in triple-negative breast cancer cells

We investigated whether deguelin is capable of inducing apoptosis in MCF-7 and MDA-MB-231 cells. After treating MCF-7 and MDA-MB-231 cells with deguelin for 3 days, typical apoptosis morphological changes were observed including membrane blebbing and chromatin condensation. Western blot analysis revealed that $10^{-6}$ M deguelin treatment for 3 days induced PARP cleavage in these cells (Fig. 2A). Flow cytometry following PI single (Fig. 2B) or Annexin V-FITC/PI double (Fig. 2C) staining revealed a potent proapoptotic for deguelin activity in these cells.

Fig. 1. The effect of deguelin on the viability and colony forming ability of MCF-7 and MDA-MB-231 breast cancer cells. (A) Breast cancer cells were treated with different concentrations of deguelin (0, 0.01, 0.1, and 1 µM) in DMEM supplemented with 10% FBS for 3 days. Cell viability was measured using an MTT assay. Each value represents the mean (±SD) of a representative of three independent experiments. **P value < 0.01; ***P value < 0.001 compared with vehicle-treated control cells. (B) An anchorage-dependent clonogenic growth assay was performed using breast cancer cell lines. Cells were treated with different deguelin concentrations (0.01, 0.1, and 1 µM) in DMEM supplemented with 10% FBS for 7–10 days, and then colonies were fixed with 0.1% coomassie blue. The number of colonies with more than 50 cells was then counted. Each value represents the mean (±SD) of a representative of three independent experiments. (C) Cells were plated in RPMI 1640 medium containing 0.5% agar overlaid on a base of 1.0% agar in culture medium. After the upper cell agar solidified, deguelin-containing medium was overlaid. After 2 weeks, colonies >125 µm in diameter were counted. Each value represents the mean (±SD) of a representative of 3 independent experiments.
3.3. Deguelin induces tumor regression in vivo

We tested the effects of deguelin on the growth of MCF-7 breast cancer cell xenograft tumors established in athymic nude mice to determine whether deguelin could induce antitumor effects on breast cancers in vivo. Mice treated with deguelin demonstrated significantly reduced tumor growth compared with control mice (Fig. 3A). At the end of the study, the tumor volume for vehicle-treated control mice increased to 629.9 ± 266.1% (mean ± SD) of the pretreatment volume, whereas that for deguelin-treated mice demonstrated only a minimal change in body weight (Fig. 3C). Together, these results suggest that deguelin inhibited breast tumor growth in the MCF-7 xenograft model.

3.4. Deguelin treatment significantly inhibits the survivin pathway

We have previously shown that Akt inactivation and decreased survivin expression contribute to the apoptotic and tumor suppressive effects of deguelin [4,9]. Hence, immunohistochemical (IHC) analysis was performed to evaluate whether deguelin treatment suppressed pAkt and survivin expression in MCF-7 xenograft tissues. Compared with the control group, deguelin-treated mice demonstrated much weaker staining for pAkt and survivin in tumor tissues (representative stains for each group are shown in Fig. 4A). To further confirm the deguelin in vivo effects, the levels of survivin and PARP cleavage were measured in tissues by Western blot analysis (Fig. 4B). The protein expression of survivin and cleaved PARP (85-kDa) was significantly lower in deguelin-treated tumors compared with the control tumors. Additionally, pERK1/2, one of the downstream effectors of the growth factor-mediated signaling pathway that is involved in the increased expression and stability of the survivin protein [13], was markedly down-regulated in the deguelin-treated tumors. These findings indicate that deguelin is a potent inducer of apoptosis in vivo in breast cancer cell xenografts.

3.5. Deguelin affects the insulin-like growth factor receptor-1 signaling pathway

We investigated the mechanism by which deguelin exhibits antitumor activities in breast cancer cells. Based on previous reports indicating the role of the IGF-1R pathway in Akt activation and survivin expression [11], we determined whether the IGF-1R pathway was involved in the deguelin-mediated antitumor effects in breast cancer cells. Western blot analysis revealed that deguelin treatment down-regulated the phosphorylation of IGF-1R and Akt in MCF-7, MDA-MB-231 and MDA-MB-468 cells (Fig. 5A). Deguelin treatment also decreased the total IGF-1R and Akt expression in MCF-7 cells, possibly due to the deguelin inhibitory effects on Hsp90 function.

Because the IGF-1R pathway is regulated by IGF-binding proteins (IGFBPs) that bind IGFs in the extracellular milieu with high affinity and reduces the IGF bioavailability [14], we also tested whether IGFBPs were implicated in the deguelin-induced regulation of the IGF-1R pathway. Western blot analysis revealed that the expression of IGFBP-3, a major IGFBP known to bind to more than 80% of circulating IGFs [15], was greatly increased in deguelin-treated MCF-7 and MDA-MB-231 cells (Fig. 5A). IGFBP-3 expression was also increased in MDA-MB-468 cells treated with deguelin (Supplementary Fig. 3). According to RT-PCR analysis, the exposure of MCF-7 and MDA-MB-231 cells to deguelin resulted in an increase in the IGFBP-3 mRNA level (Fig. 5B). In contrast, IGFBP-2 expression was markedly decreased in MCF-7 treated (Supplementary Fig. 3) and was undetectable in MDA-MB-468 cells (Supplementary Fig. 3) and was undetectable in MDA-MB-231 cells (Fig. 5A).

We next analyzed whether the pIGF-1R and IGFBP-3 expression levels were modulated by deguelin treatment in vivo. We performed immunohistochemical analysis on tissues derived from MDA-MB-231 breast cancer cell xenograft tumors established in athymic nude mice. Tumors from mice treated with deguelin demonstrated reduced pIGF-1R along with increased IGFBP-3 expression (Supplementary Fig. 3). According to RT-PCR analysis, the expression of IGFBP-3 in deguelin-treated tumors compared with the control tumors. Additionally, pERK1/2, one of the downstream effectors of the growth factor-mediated signaling pathway that is involved in the increased expression and stability of the survivin protein [13], was markedly down-regulated in the deguelin-treated tumors. These findings indicate that deguelin is a potent inducer of apoptosis in vivo in breast cancer cell xenografts.
of an IGFBP-3 shRNA or IGF-1R expression vector, respectively. The knockdown of IGFBP-3 in MCF-7 cells attenuated the effects of deguelin on anchorage-dependent colony formation and IGF-1R phosphorylation (Fig. 6A and data not shown). In addition, IGF-1R overexpression also counteracted the deguelin effects on colony formation and pIGF-1R expression (Fig. 6B). These results suggest that deguelin exerts antiproliferative activities in breast cancer cells regardless of their ER, PR, and HER2 expression status, at least in part due to its inhibitory effects on the IGF-1R pathways via its impact on the expression of IGFBP-3 and a number of IGF-1R signaling components including Akt and ERK.

4. Discussion

Although patients with steroid receptor-positive breast cancer have been successfully treated with antiestrogens and aromatase inhibitors, patients with ER-, PR-, and triple-negative breast cancers have limited therapeutic options available. In this study, we have demonstrated, to our knowledge for the first time, that the phytochemical deguelin suppresses the growth of breast cancer cells by down-regulating IGF-1R signaling activation at least in part by controlling IGFBP-3 expression.

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It is generally accepted that the IGFs exert tumorigenic actions mainly through the type 1 IGF receptor (IGF-1R) [16,17]. Activated IGF-1R provides a critical cell survival signal that protects against apoptosis by activating a number of downstream effectors including Akt [18], which plays a key role in cell cycle progression, apoptosis inhibition, and angiogenesis stimulation [19]. The dysregulation of the IGF system is well recognized as a key contributor to the progression of multiple cancers. Furthermore, the anti-apoptotic properties of IGF-1R confer the resistance to chemotherapy and radiotherapy for cancer cells. The overexpression of the IGF-1R has been observed in most breast cancer cell lines regardless of their ER status and estrogen responsiveness [20] and in a large series of human tissue specimens [21,22]. These findings provide a rationale for targeting IGF-1R as an anticancer therapeutic strategy. Several approaches with a major focus on monoclonal antibodies (mAbs) and small molecules that block IGF-1R have been investigated as anticancer therapeutic strategies [23]. The potential advantage of mAbs is their ability to bind IGF-1R and IGF-1/IR hybrid receptors without affinity for IR; however, growing evidence has implicated IR signaling as a key player in growth and proliferation. In this setting, another approach using small molecular IGF-1R tyrosine kinase inhibitors (TKIs) has the potential advantage of inhibiting both IGF-1R and IR although the potential...
significant inhibits Akt activation and the expression of survivin, one of the inhibitor of apoptosis (IAP) family members that is frequently overexpressed in a variety of human cancers [9,30]. When tested in vivo in a variety of animal models, prolonged deguelin administration was well tolerated without detectable organ or systemic toxicity [5]. Mechanistically, deguelin interferes with Hsp90 function and induces the degradation of a variety of Hsp90 client proteins, including Akt and HIF-1α [4,6,31,32], decreases COX-2 expression, and induces AMPK activation [9,30]. It has recently been demonstrated that deguelin effects on GSK-3β dephosphorylation cause the restoration of its ability to prime β-catenin degradation, resulting in the destabilization of free β-catenin, which is a critical step in Wnt signaling in cancer cells [33]. Previously, in vitro findings demonstrated the ability of deguelin to abrogate Akt activation and survivin expression and induce apoptosis in triple-positive MCF-7 and HER2-positive SK-BR-3 breast cancer cells but not in normal mammary epithelial cells [11]. In this study, we demonstrate the apoptotic activities of deguelin in the triple-negative MDA-MB-231 and MDA-MB-468 breast cancer cells and its suppressive effects on the growth of MCF-7 cell xenograft tumors. Our current results further demonstrate that the deguelin inhibitory effects on the expression of IGF-1R and Akt in MCF-7 but not MDA-MB-231 cells. Given the capacity of deguelin to inhibit Hsp90 function [32], the regulation of these Hsp90 clients by deguelin is not unexpected. The different levels of client protein regulation by deguelin treatment may depend on the intracellular activity of the various E3 ubiquitin ligases and/or deubiquitylases and deguelin metabolism.

Of particular interest is the deguelin-induced transcriptional up-regulation of IGFBP-3, a major IGFBP that modulates the bio-availability of both IGF-1 and IGF-2 [15]. IGFBP-3 is also known to induce apoptosis through IGF-independent mechanisms. Although the potential of IGFBP-3 as a therapeutic target for antineoplastic therapies has been shown in a variety of human cancers [32,34–38], it is known to potentiate IGF action under certain circumstances [39]. In view of these observations, it is possible that IGFBP-3 can negatively influence apoptotic pathways in breast cancer cells. However, previous findings indicate that (1) rhIGFBP-3 attenuates the anti-apoptotic effects of IGF-I but not that of the long R3 IGF-I [22], (2) exogenous IGFBP-3 treatment inhibits the growth of a number of breast cancer cells, including MCF-7 and Hs578T [40], (3) IGFBP-3 mediates the antiproliferative effects of TGFβ2 and retinoic acid, a potent antitumor factor in ER− and ER+ human breast cancer cells [41,42], and (4) IGFBP-3 expression is frequently reduced in breast cancer [43]. These findings support the role of IGFBP-3 as an inhibitor of cell growth in the biology of human breast cancer cells. Our observation that IGFBP-2 expression, which has been demonstrated to increase the invasive capacity of glioblastoma and ovarian cancer cells [44,45], suggests that IGFBP-2 regulation may provide another mechanism by which deguelin regulates breast cancer cells. These collective findings suggest that the deguelin antitumor activities in breast cancer cells work through multiple mechanisms including the modulation of the IGFBP-2 and -3 expression, the suppression of the IGF-1R signaling pathway, and IGF-independent IGFBP activities.

In summary, our studies provide several important findings, including the following: (1) deguelin treatment at concentrations less than 1 μM, which is achievable in mice given a single oral dose of deguelin at 4 mg/kg [5], inhibits the proliferation of breast cancer cells by inducing apoptosis regardless of the ER, PR and HER2 expression status; (2) the systemic administration of deguelin (4 mg/kg) suppresses the growth of MCF-7 cell xenograft tumors in vivo; and (3) the deguelin antitumor activities in breast cancer cells work at least in part through the regulation of IGF-1R pathways via a transcriptional increase in IGFBP-3 gene expression in
addition to its impact on Hsp90 function [6]. Given all of the previous and current results and the redundancy and complexity of the molecular abnormalities present in most breast cancers, the capacity of deguelin capacity to alter the activity of multiple oncogenic targets provides a rationale for the use of deguelin as a promising antineoplastic strategy capable of combating breast cancer. Additional preclinical and clinical trials may prove the unique therapeutic benefits of deguelin. Further investigation is also warranted to identify the underlying mechanisms for the deguelin-mediated regulation of IGFBP-2 expression.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.canlet.2013.01.022.

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