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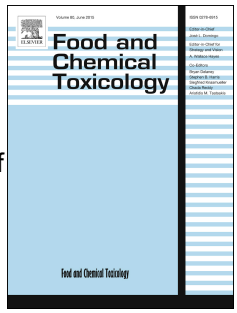

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Genipin inhibits the invasion and migration of colon cancer cells by the suppression of HIF-1α accumulation and VEGF expression

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Running title: Genipin induced suppression of HIF-1α accumulation

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Abstract

Hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) play important roles in cancer progression in various cancer cell lines. Although genipin, a constituent of Gardenia fruit, has been shown to have anti-tumor activity, its role in the suppression of HIF-1 and its downstream target genes is not well understood. We examined the effect of genipin on the intracellular level of HIF-1α and extracellular level of VEGF using the colon cancer cell line HCT116. We observed that genipin suppressed the accumulation of HIF-1α under hypoxia in various cancer cell lines, including HCT116, via the modulation of protein degradation. Genipin also suppressed the expression of VEGF and the invasion of colon cancer cells by blocking the extracellular signal-regulated kinase signaling pathway. Taken together, our results provide new insights into the potential role of genipin in suppressing colon cancer progression.

Keywords: Genipin, Hypoxia-inducible factor-1, Vascular endothelial growth factor, Invasion, Akt, ERK

1. Introduction

Although treatment outcomes have improved since the development of molecular targeted agents in patients with metastatic colon cancer, there is still an unmet need for the development of therapeutic agents owing to the limitations of current treatments.

Genipin, a metabolite derived from geniposide, is present in the fruit of *Gardenia jasminoides* Ellis. Previous studies have identified various roles of genipin, e.g., as an anti-inflammatory (Nam et al., 2010), anti-angiogenic (Park et al., 2003), anti-oxidative (Wang et al., 2009), and anti-thrombotic (Suzuki et al., 2001) agent. In addition, a potential of genipin as an anti-neoplastic agent has been suggested based on its ability to induce apoptosis and suppress metastasis in various human cancer cell lines (Cao et al., 2010; Kim et al., 2005; Wang et al., 2012) (Feng et al., 2011) (Hong and Kim, 2007).

Hypoxia, which is a common feature of the tumor cell environment in various cancers, induces the activation of hypoxia-inducible factor-1 (HIF-1), a heterodimeric transcription factor. HIF-1 is composed of two transactivation domains, the HIF-1α and HIF-1β subunits. The α subunit usually forms a complex with the von Hippel-Lindau tumor suppressor protein, leading to degradation via the ubiquitin-proteasome process under normoxic conditions. The stability of HIF-1α under hypoxia is controlled by several pathways, resulting in the accumulation of HIF-1α and dimerization with HIF-1β. The HIF-1α/β dimer subsequently translocates to the nucleus and triggers the expression of downstream genes (Hicklin and Ellis, 2005; Iliopoulos et al., 1996; Jaakkola et al., 2001; Maxwell and Ratcliffe, 2002; Maxwell et al., 1999).

Vascular endothelial growth factor (*VEGF*), a downstream gene activated by HIF-1, plays a salient role in angiogenesis. Signaling by the interaction between VEGF and its receptor,

VEGFR, promotes tumor growth and progression via the invasion, migration, proliferation, and activation of endothelial cells and increased microvascular permeability (Choong and Nadesapillai, 2003; Hicklin and Ellis, 2005; Zachary and Gliki, 2001). VEGF is associated not only with endothelial cell migration and invasion, which are crucial steps in the early phase of angiogenesis, but also with cancer cell invasion by inducing the expression of matrix metalloproteinases (MMPs), urokinase plasminogen activator (uPA) and its receptor (uPAR), and tissue-type plasminogen activator (Choong and Nadesapillai, 2003; Hicklin and Ellis, 2005; Krishnamachary et al., 2003; Zachary and Gliki, 2001). A previous study has reported that hypoxia-induced HIF-1α overexpression stimulates the Matrigel invasion of colon cancer cells and triggers the expression of proteins involved in the pathophysiology of invasion (Krishnamachary et al., 2003). Another study has also demonstrated that HIF-1α accumulation and VEGF expression via Akt/phosphoinositide 3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) 1/2 activation play a role in regulating hepatoma and tongue cancer cell invasion (Zhang et al., 2005).

Despite abundant data related to the role of genipin in apoptosis, its anti-invasive mechanism in cancers has been scarcely clarified. The activation of c-Jun NH2-terminal kinase (JNK) is a well-known mechanism underlying genipin-induced apoptosis in various cancer cells, including breast cancer, prostate cancer, hepatoma, and cervical cancer cells (Cao et al., 2010; Hong and Kim, 2007; Kim et al., 2005; Kim et al., 2012). The induction of apoptosis by genipin is also thought to play an important role in suppressing hematologic malignancies (Feng et al., 2011; Lee et al., 2011). On the other hand, only a few studies have reported the effect of genipin on the inhibition of cancer invasion. In hepatocellular carcinoma, the p38/TIMP-1/MMP-2 pathway is involved in the anti-metastatic effect of genipin (Wang et al., 2012). The inhibition of cancer invasion by genipin has also been

88 confirmed in breast cancer cells via unclarified pathways (Gupta et al., 2013).

89 Given that one of suggested mechanisms underlying genipin-mediated anti-invasive activity
90 was the inhibition of MMP-2 via the p38/TIMP-1/MMP-2 pathway, we thought it would be
91 valuable to examine another possible mechanism for the inhibition of cancer invasion by
92 genipin. Many previous results have shown that hypoxia-induced HIF-1 and VEGF are
93 associated with the increased expression of MMP in cancer invasion (Gupta et al., 2013;
94 Krishnamachary et al., 2003; Lamoreaux et al., 1998; Zachary and Gliki, 2001); accordingly,
95 we investigated if genipin exerts anti-invasive activity by the inhibition of the HIF-1/VEGF-
96 mediated expression of MMP in colon cancer cells. Here, our results provide important
97 information regarding the mechanism by which genipin inhibits cancer progression using a
98 human colon cancer cell line.

99

100 **2. Materials and methods**

101 2.1. Cell lines

102 Human colon cancer (HCT116 and HT29), human breast cancer (SKBR-3), and human
103 prostate cancer (DU145) cell lines were purchased from the Korea Cell Line Bank. To test for
104 mycoplasma contamination in all cells, we used the MycoAlert™ PLUS Mycoplasma
105 Detection Kit (Lonza, Allendale, NJ, USA). Human breast cancer SKBR-3 cells were
106 cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA). Human prostate cancer
107 DU145 cells and colon cancer HT29 cells were cultured in Dulbecco's modified Eagle's
108 medium (Gibco BRL, Gaithersburg, MD, USA). HCT116 colon cancer cells and HepG2 liver
109 cancer cells were cultured in McCoy's 5A medium (Invitrogen, Carlsbad, CA, USA). The
110 human colon cell line CCD-18Co and normal lung cell line Beas 2B were purchased from the

ATCC (Manassas, VA, USA). All cancer cell lines were cultured with 70% fetal bovine serum (Hyclone, Logan, UT, USA) and 26 μM sodium bicarbonate for monolayer cell culture under a humidified atmosphere of 5% CO_2 in air at 37°C.

2.2. Hypoxia and treatment with reagents

Genipin, 99% purity, was purchased from Sigma-Aldrich (St. Louis, MO, USA) or Selleckchem (Houston, TX, USA). Dimethyl sulfoxide (DMSO) was employed to increase genipin solubility, which is greater in organic solvents than in phosphate-buffered saline. Cycloheximide (CHX) and deferoxamine (DFX) were obtained from Calbiochem (San Diego, CA, USA). Cells on Petri dishes were incubated in a hypoxic chamber (Forma Scientific, Marietta, OH, USA) with a 94:5:1 mixture of $N_2/CO_2/O_2$ for hypoxia treatment. Deoxygenated media were manufactured by equilibration with a hypoxic gas mixture containing 5% CO_2, 94% N_2, and 1% O_2 at 37°C before each experiment. After reaching 70-80% confluence, cells were prepared in complete media and treated with CHX and various concentrations of genipin. Continual incubation was performed in normal culture conditions under hypoxia exposure (1% O_2) during the indicated time intervals.

2.3. Survival assay

Cells were treated with MTS reagent for 3 h at 37°C in an atmosphere of 5% CO_2, and cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Roche Life Science, San Francisco, CA, USA). Absorbance at 450 nm was determined using an enzyme-linked immunosorbent assay (ELISA) plate reader.

2.4. Immunoblot assay

A western blot analysis was performed according to a protocol described previously (Gupta

et al., 2013). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% non-fat dry milk in phosphate-buffered saline-Tween-20 (0.1%, v/v) for 1 h. The diluted primary antibodies were incubated with the membrane at 4°C for 1 h, according to the manufacturers' instructions. Monoclonal antibodies were purchased from the following companies: anti-HIF-1α from BD Biosciences (San Jose, CA, USA), anti-actin from ICN (Costa Mesa, CA, USA), and anti-Akt, anti-phospho-Ser^{473}-Akt, anti-HSP90, anti-ERK, anti-phospho-ERK, anti-MMP-2, anti-MMP-9, and anti-uPA from Cell Signaling Technology (Beverly, MA, USA). Horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG was used as the secondary antibody. Chemiluminescence (ECL; Amersham, Arlington Heights, IL, USA) was used for the visualization of immunoreactive proteins. Experiments were repeated at least three times for the immunoblotting assays. Densitometric analysis was performed using a gel image analysis program, ImageJ (NIH, Bethesda, MD, USA).

2.5. Transcriptional activation assay

A luciferase assay was carried out as described previously (Emerling et al., 2008). Cells were transiently transfected with a luciferase reporter plasmid pGL2-hypoxia-response element (HRE) and a reference luciferase plasmid pRL-CMV (Promega, Madison, WI, USA) with FuGENE HD (Roche Diagnostics), according to the manufacturer's instructions.

2.6. Real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD, USA) according to the manufacturer's instructions. TaqMan primers from Life Technologies Applied Biosystems (Foster City, CA, USA) were purchased and used to measure gene

expression (*HIF-1α*, hypoxia-inducible factor-1: Hs00153153_m1; *VEGF*, vascular endothelial growth factor: Hs00900055_m1; *GLUT1*, glucose transporter 1: Hs00892681_m1; *CA9*, carbonic anhydrase 9: Hs00154208_s1; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase: Hs03929097_g1). RT-PCR was performed using gene-specific TaqMan probes on an Applied Biosystems StepOnePlus Real-Time PCR system with TaqMan PCR Master Mix (Life Technologies).

2.7. ELISA

HCT116 cells (1×10^5) were plated on a 60-mm plate in RPMI medium and incubated overnight before treatment. The cell culture medium was removed and stored at –80°C. Determination of VEGF levels in the media was carried out by ELISA using a commercial kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol, as described previously (Gupta et al., 2013).

2.8. Invasion assay

Twenty-four-well BD BioCoat Growth Factor-Reduced Matrigel Invasion Chambers (BD Biosciences, Bedford, MA, USA) were used for Transwell invasion experiments. The invasion chambers consisted of a BD Falcon® cell culture insert with an 8-μm pore size polyethylene terephthalate (PET) membrane coated with BD Matrigel Matrix, which serves as a reconstituted basement membrane *in vitro*. HCT116 cells exposed to hypoxia were prepared for the cell suspension in serum-free culture medium at 1×10^5 cells without or with genipin according to the concentration gradient. Membranes with cells attached to the lower membrane surface (invaded cells) were fixed with methanol and stained with hematoxylin. Invasiveness was determined by counting the number of invaded cells under a microscope at 100x magnification. Results are presented as the average number of cell per filter of triplicate

179 experiments.

180 2.9. Statistical analysis

181 Statistical significance was calculated using Student's *t*-tests. The two-sample *t*-test was
182 used for two-group comparisons. Values are reported as means ± standard deviation (SD). A
183 value of $p < 0.05$ was considered significant.

184

185 **3. Results**

186 *3.1. Genipin inhibits the accumulation of HIF-1α under hypoxia in human colon cancer cells*

187 Genipin is a chemical compound with anti-tumor activity (Fig. 1A) (Cao et al., 2010; Feng
188 et al., 2011; Hong and Kim, 2007; Kim et al., 2005; Roux and Blenis, 2004). We investigated
189 the effects of co-treatment with genipin and hypoxia on the viability of normal cell lines
190 (colon and lung). We found that co-treatment with genipin and normoxia or hypoxia does not
191 induce cell death in either cell line (Fig. 1B). To determine whether genipin exerts a cytotoxic
192 effect in colon cells by inhibiting HIF-1α accumulation, an MTS assay was performed using
193 HCT116 colon cancer cells treated with various concentrations of genipin under hypoxic
194 conditions. Cell viability was measured in an environment of 1% oxygen with 5 to 200 μM
195 genipin. Decreased viability was observed after 24 h of incubation with 200 μM genipin (Fig.
196 1C). We found no significant change in colon cancer cell viability after treatment with 50 μM
197 genipin for 8 h (Fig. 1C), but HIF-1α protein accumulation increased after exposure to
198 hypoxia for 8 h (Fig. 1D). Furthermore, HIF-1α accumulation as well as the phosphorylation
199 of Akt were suppressed by 50 μM genipin (Fig. 1E), suggesting that genipin had a minimal
200 effect on viability in HCT116 cells. The suppression of HIF-1α accumulation was also

observed in various cancer cells, such as liver, prostate, colon, and breast cancer, treated with a low concentration (50 μM) of genipin under hypoxia (Fig. 2A–D).

3.2. Genipin-induced suppression of HIF-1α accumulation via the modulation of protein degradation

An HRE promoter-luciferase reporter assay using HCT116 cells was performed to evaluate the transcriptional activity of *HIF-1α* in response to hypoxia after treatment with genipin. Decreased luciferase activity was observed in cells treated with 50 μM genipin under hypoxia (Fig. 3A) as well as in cells treated with a combination of 50 μM genipin and DFX, another known HIF-1 inducer, under normoxic conditions (Fig. 3B). We did not observe a change in the mRNA expression of *HIF-1α* after genipin treatment under hypoxia, but decreased mRNA expression levels of other hypoxic markers, such as *VEGF*, *GLUT1*, and *CA9*, were observed (Fig. 3C). Because genipin had little effect of the mRNA expression of *HIF-1α*, we evaluated if genipin modulates protein expression. We observed that suppressed HIF-1α protein expression was more prominent in HCT116 cells treated with a combination of genipin and CHX than in those treated with CHX alone under hypoxia (Fig. 4A, B). Suppressed HIF-1α accumulation was also observed in colon cancer cells treated with a combination of MG132 and genipin (Fig. 4C), suggesting that genipin is involved in the modulation of HIF-1α protein degradation.

3.3. Genipin leads to the suppression of VEGF expression and invasion of colon cancer cells by inhibiting the ERK signaling pathway

Given that VEGF is a downstream target of HIF-1α (Hicklin and Ellis, 2005), ELISA was performed to determine the extracellular levels of VEGF in the presence of genipin. We found that the expression of VEGF decreased as the concentration of genipin increased under

hypoxia (Fig. 5A). Because VEGF induces the expression of proteins involved in cell invasion, such as MMP and uPA (Choong and Nadesapillai, 2003; Ferrara and Davis-Smyth, 1997; Hicklin and Ellis, 2005), we evaluated the role of genipin in the migration and invasion of colon cancer cells. Dose-dependent inhibition of cancer cell invasion was detected in HCT116 cells treated with genipin under hypoxia by an invasion assay (Fig. 5B). We next examined the expression levels of MMP-2, MMP-9, and uPA by western blotting in genipin-treated HCT116 cells under hypoxia. Reduced expression of these proteins was confirmed in the presence of genipin. Interestingly, the expression level of phosphorylated ERK was also decreased by treatment with genipin, suggesting that the genipin-induced suppression of invasion could be mediated by blocking the ERK signaling pathway (Fig. 5C, D).

4. Discussion

Owing to the limited number of effective therapeutic agents for metastatic colon cancer, there has been a persistent need for the development of novel agents. HIF-1 is involved in cancer progression by promoting angiogenesis and metastasis, and these effects are mediated by VEGF under hypoxia (Hicklin and Ellis, 2005). Although targeting HIF-1 has been of great interest given its significant role in tumor progression, no clinically available HIF-1 inhibitors have been developed. However, several studies have suggested that chemoprevention may be possible using natural products, such as phenethyl isothiocyanate, apigenin, or chrysin, in various cancers by showing the suppression of HIF-1α and VEGF (Emerling et al., 2008; Fang et al., 2007; Fu et al., 2007; Gupta et al., 2013).

Genipin has also been regarded as a potential chemopreventive natural product based on its cancer suppressive effect. Previous studies have demonstrated that the metabolic pathway of

247 genipin is a benign crosslinking agent that strengthens the mechanical properties of tissues
248 (Reich and Akkus, 2013). Another study has found genipin sulfate as a major metabolite of
249 geniposide; however, it may not be permeable through the intestinal membrane and seems
250 indispensable to be hydrolyzed to lipophilic genipin in the intestinal lumen before absorption
251 (Akao et al., 1994; Hou et al., 2008). Here, we showed that genipin inhibits the accumulation
252 of HIF-1α under hypoxia in various human cancer cell lines, including colon cancer. The
253 suppression of HIF-1α as well as proteins involved in cancer invasion was accompanied by
254 the decreased expression of phospho-Akt and phospho-ERK, suggesting that the detrimental
255 effect of HIF-1α was mediated by the Akt/PI3K and ERK 1/2 signaling pathways. New vessel
256 formation is a critical process in tumor progression, and targeting signaling by VEGF and its
257 receptor is one of the most important treatment options for several cancers. Given the
258 inhibitory effect of genipin on VEGF, genipin might act on colon cancer cells to inhibit
259 angiogenesis synergistically with agents targeting VEGF or VEGFR. In addition, since Akt
260 and ERK1/2 are downstream signaling mediators of epidermal growth factor receptor
261 (EGFR), it is possible that genipin has an inhibitory effect on EGFR. Monoclonal antibodies
262 targeting EGFR are another core axis in the treatment of colon cancer, along with anti-
263 angiogenesis agents; accordingly, further studies on the possible role of genipin in blocking
264 EGFR activation might provide another potential novel therapeutic agent in colon cancer.

265 Interestingly, the inhibitory activity of genipin on HIF-1 was not mediated by the
266 suppression of transcription, but by the modulation of protein degradation. In addition, our
267 results showed that genipin inhibited HIF-1α activity both under hypoxia and DFX treatment,
268 suggesting a highly specific property of genipin against HIF-1α, regardless of the hypoxic
269 condition, i.e., physiological or chemical (Chau et al., 2005).

270 These findings provide new insights into mechanisms by which genipin suppresses the

invasive capacity of colon cancer cells. Genipin inhibited HIF-1α accumulation and VEGF expression, subsequently resulting in the decreased expression of MMP and uPA, proteins associated with basement membrane degradation, under hypoxia. HIF-1α and VEGF have been reported to be biomarkers for the advanced stage of colon cancer. A previous study has reported that metastasis of colon cancer cells to the liver is significantly associated with the expression of HIF-1α and VEGF, as indicated by immunohistochemical staining of tissues from patients with colon cancer (Cao et al., 2009). In addition, many *in vitro* studies have highlighted the role of HIF-1 in promoting metastasis of colon cancer cells (van der Bilt et al., 2007; Zhang et al., 2014; Zhao et al., 2010). Although many natural products have been shown to have the inhibitory effect on cancer migration, the inhibitory mechanism for genipin has not been clarified. Our previous study demonstrated that Akt and ERK inhibitors have synergistic effects with a natural product in inactivating those proteins (Gupta et al., 2013), suggesting that the anti-cancer effect of genipin combined with other targeted agents should be explored further.

In summary, we presented clear results suggesting a novel role of genipin in the suppression of cancer progression using a colon cancer cell line. Genipin inhibited hypoxia- and DFX-induced HIF-1α accumulation and VEGF expression via the modulation of protein degradation. Genipin also inhibited cancer invasion as indicated by the suppressed expression of MMP and uPA. The Akt/PI3K and ERK 1/2 signaling pathways were involved in the suppression of HIF-1α expression and cancer invasion.

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298

299 **Conflicts of interest**

300 The authors declare no conflicts of interest.

301

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413

414 **Figure legends**

415 **Fig. 1.** Effect of genipin under hypoxia on the intracellular level of HIF-1α in HCT116 cells.
416 (A) Structure of genipin. (B) Effect of genipin on the viability of normal cells was determined
417 by MTS assays. The normal cell lines (CCD-18Co (colon) and Beas 2B (lung)) were treated
418 with 50 μM genipin for 24 h. (C) Effect of genipin on the viability of HCT116 cells was
419 determined by MTS assays. Error bars represent standard errors of the mean (SEM) from
420 three independent experiments. (D) Kinetics of HIF-1α protein accumulation under hypoxia.
421 (E) Dose-dependent inhibition of HIF-1α protein accumulation and Akt phosphorylation after
422 treatment with various concentrations of genipin. Beta-actin was used as a loading control.

423

424 **Fig. 2.** Effect of genipin on HIF-1α protein accumulation under hypoxic conditions in various
425 cancer cell lines. (A) HepG2, (B) DU145, (C) HT29, and (D) SkBr3 cells were cultured
426 under hypoxic conditions alone or were treated with 50 μM genipin and cultured under
427 hypoxia. Cell lysates with equal amounts of proteins were loaded and separated by SDS-
428 PAGE and immunoblotted with an anti-HIF-1α antibody. Beta-actin was used as a loading
429 control.

430

431 **Fig. 3.** Measurement of the transcriptional activity and mRNA expression of *HIF-1α*. (A) The
432 transcriptional activity of *HIF-1α* was measured by the luciferase assay after treatment with

various concentrations of genipin under hypoxia. Luciferase activity is presented as the mean ± SD of three independent experiments. Asterisks indicate statistically significant differences in mean values compared to untreated HCT116 cells (*$p < 0.05$, **$p < 0.001$). (B) The transcriptional activity of *HIF-1α* was measured after treatment with genipin (left) and DFX (right) under normoxic conditions. Error bars represent SEM from three independent experiments. The asterisk indicates a statistically significant difference compared to untreated HCT116 cells (*$p < 0.05$). (C) mRNA expression levels of *HIF-1α* and various hypoxic markers were measured by RT-PCR under normoxia (left) and hypoxia (right) in the presence or absence of genipin. Error bars represent SEM from three independent experiments. A statistically significant difference is indicated by an asterisk (*$p < 0.05$).

Fig. 4. Time-dependent effect of CHX and genipin on the accumulation of HIF-1α under hypoxia in HCT116 colon cancer cells. (A) HCT116 cells were treated with CHX alone (left) or CHX combined with genipin (right) under hypoxia. (B) Time-dependent expression of HIF-1α after treatment with CHX alone or CHX combined with genipin under hypoxia. The lower panel shows HIF-1α signal levels quantified by densitometry, and values are expressed as percentage expression relative to that of untreated cells at the indicated time points. Error bars represent SEM from three independent experiments. The asterisk indicates a statistically significant difference (*$p < 0.05$). (C) Dose-dependent inhibition of HIF-1α accumulation after treatment with various concentrations of genipin with or without MG132 under hypoxia. Beta-actin was used as a loading control.

Fig. 5. The mechanism underlying the anti-invasive effect of genipin on HCT116 colon

cancer cells. (A) Suppression of extracellular VEGF expression by genipin in a dose-dependent manner under hypoxia was measured by ELISA. Results are presented by the mean values of VEGF concentrations, and error bars represent SEM from three independent experiments. (B) Invasion parameters are indicated as means ± SD of three separate experiments. Statistically significant differences were determined by Student's *t*-tests. The mean values denoted by asterisks indicate significant differences compared to the matched HCT116 cells without treatment with genipin (*$p < 0.05$, **$p < 0.01$). (C) The expression levels of invasion-related proteins and p-ERK 1/2 relative to that of actin are indicated as means ± SD of three independent experiments. The mean values denoted with an asterisk and sharp indicate statistically significant difference compared to the matched normoxic control and hypoxic control, respectively. Statistical significance was determined by Student's *t*-tests (*$p < 0.05$, #$p < 0.001$). (D) Decreased expression of invasion-related proteins and p-ERK 1/2 after treatment with genipin under hypoxia based on an immunoblotting assay. Beta-actin was used as a loading control.

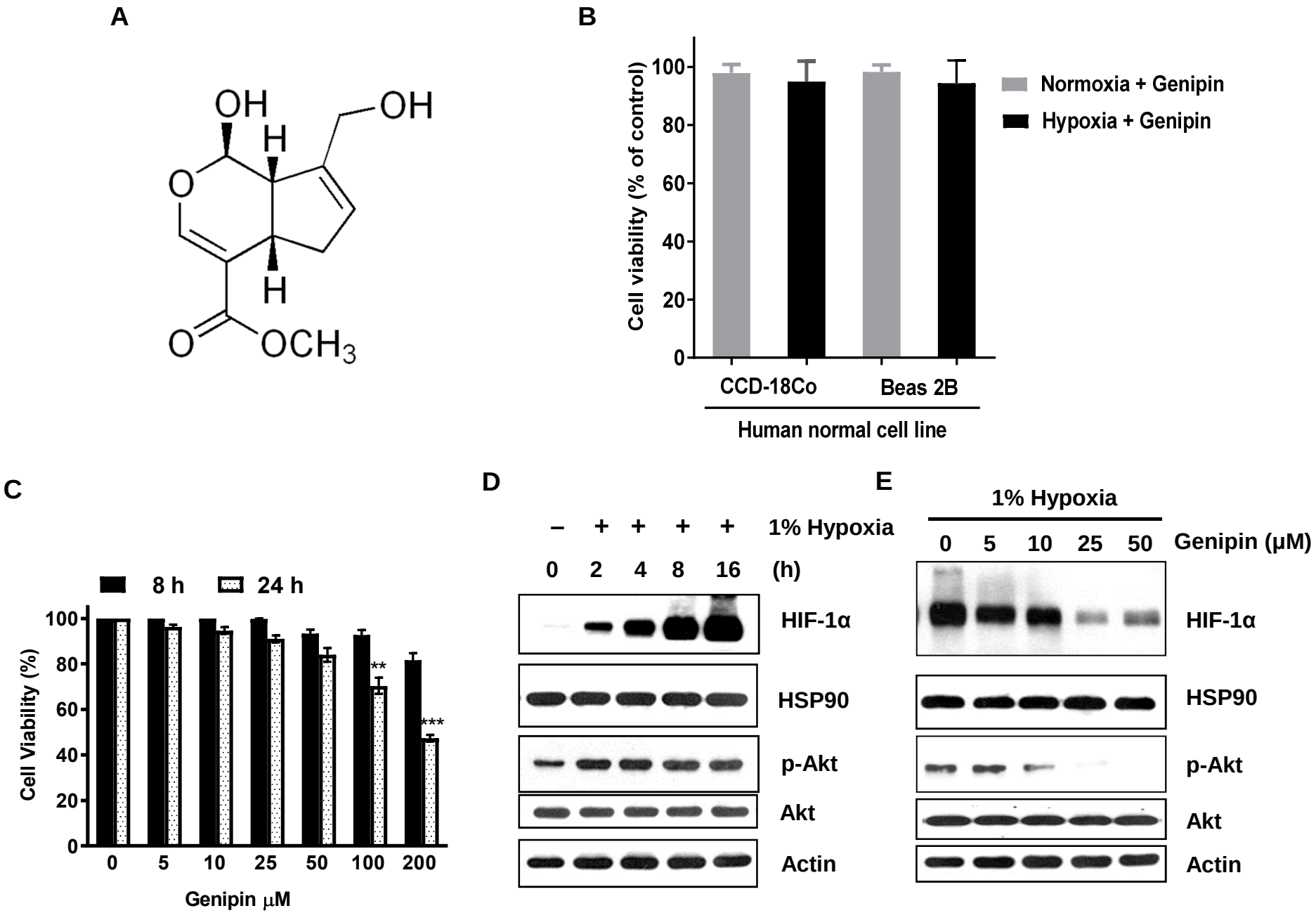

Fig. 1A, 1B, 1C, 1D, and 1E

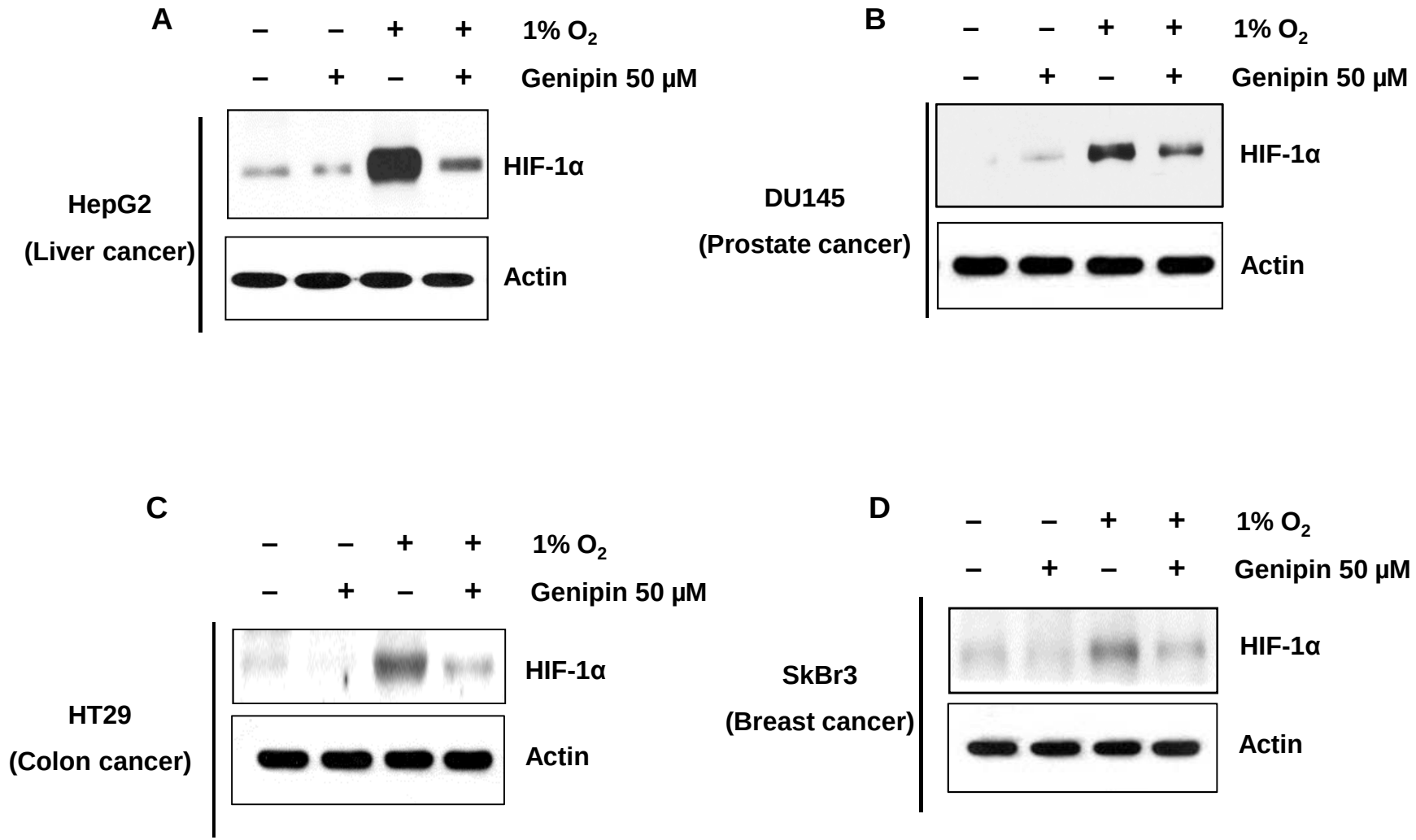

Fig. 2A, 2B, 2C, and 2D

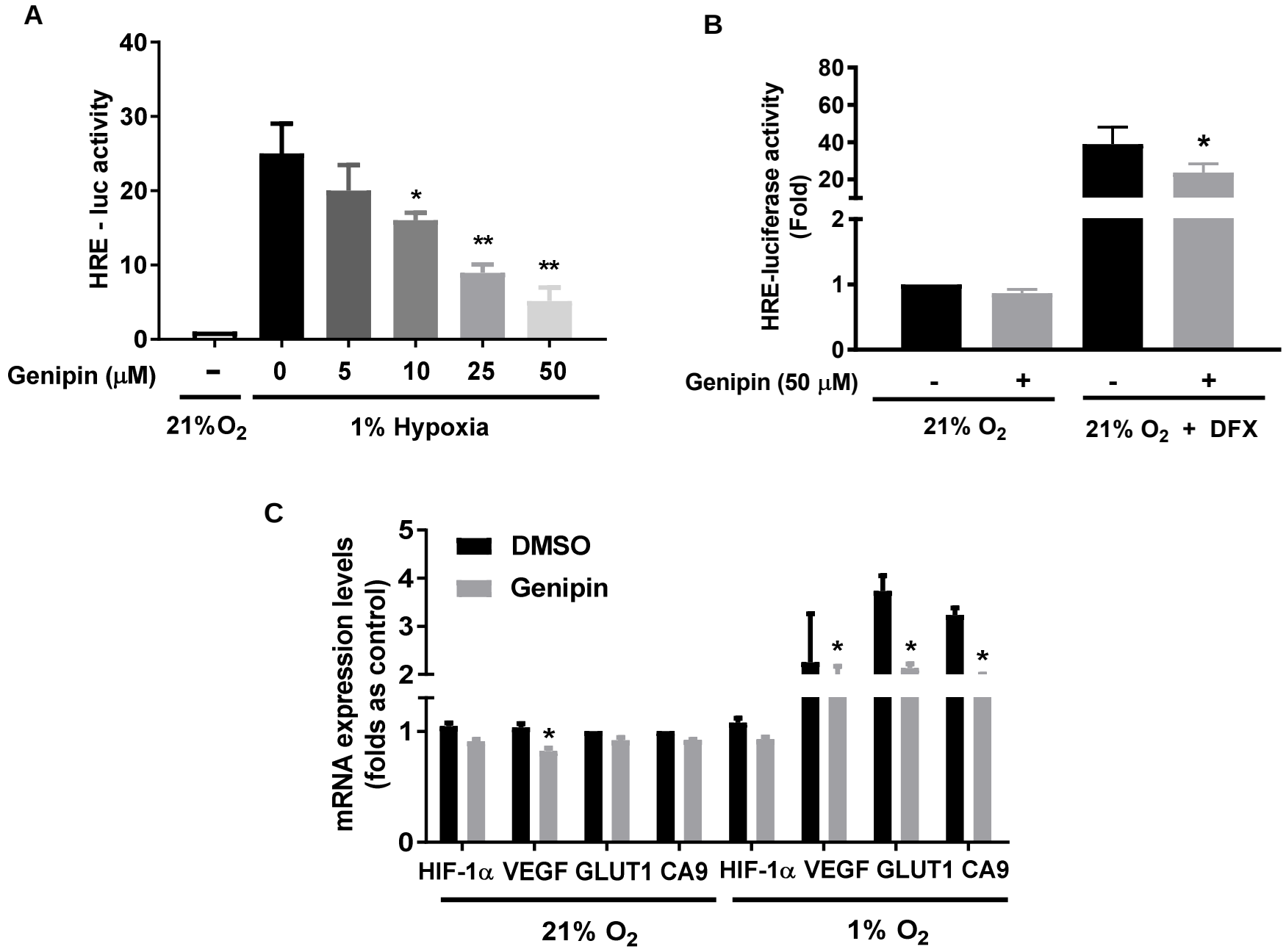

Fig. 3A, 3B, and 3C

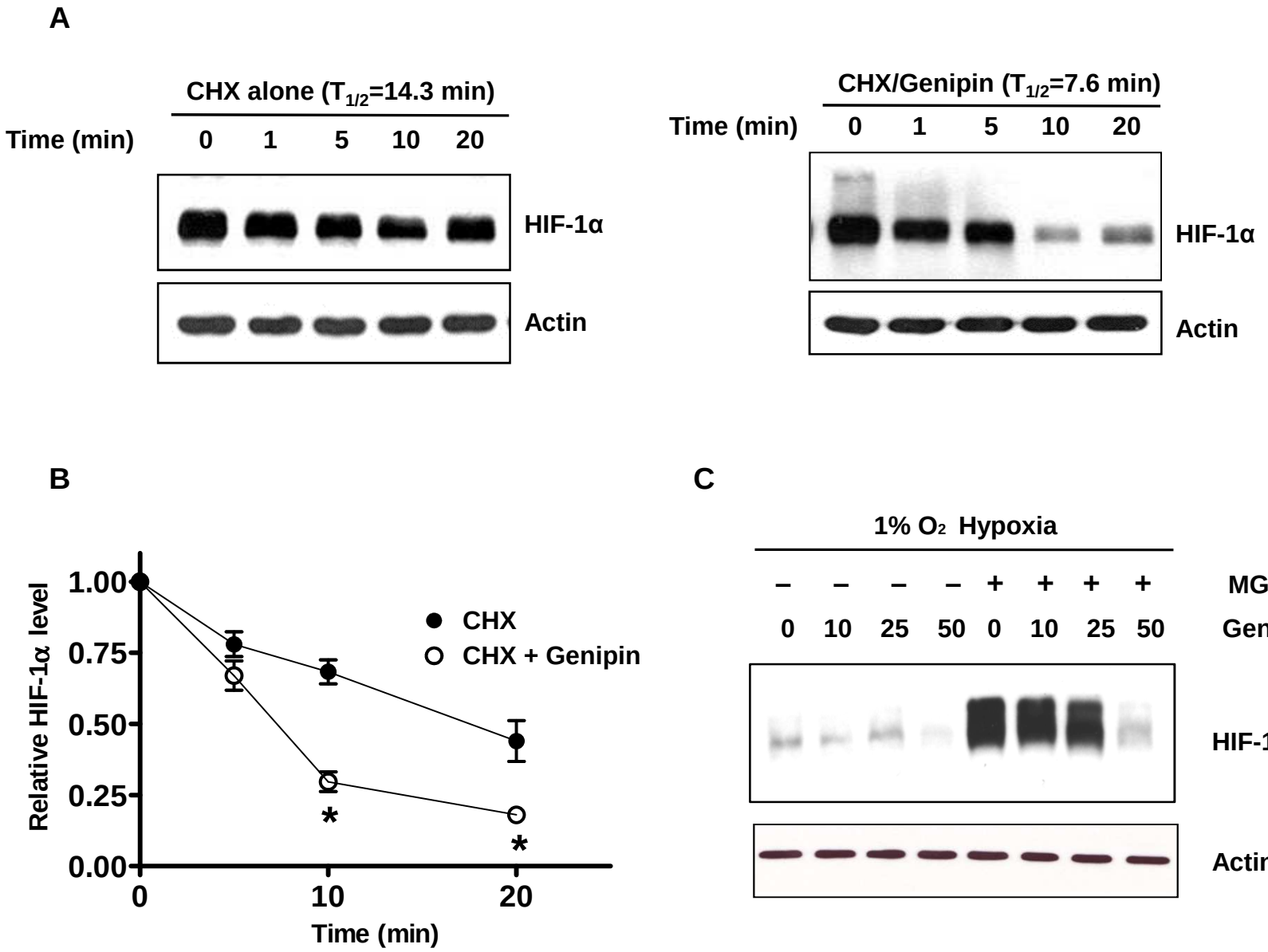

Fig. 4A, 4B, and 4C

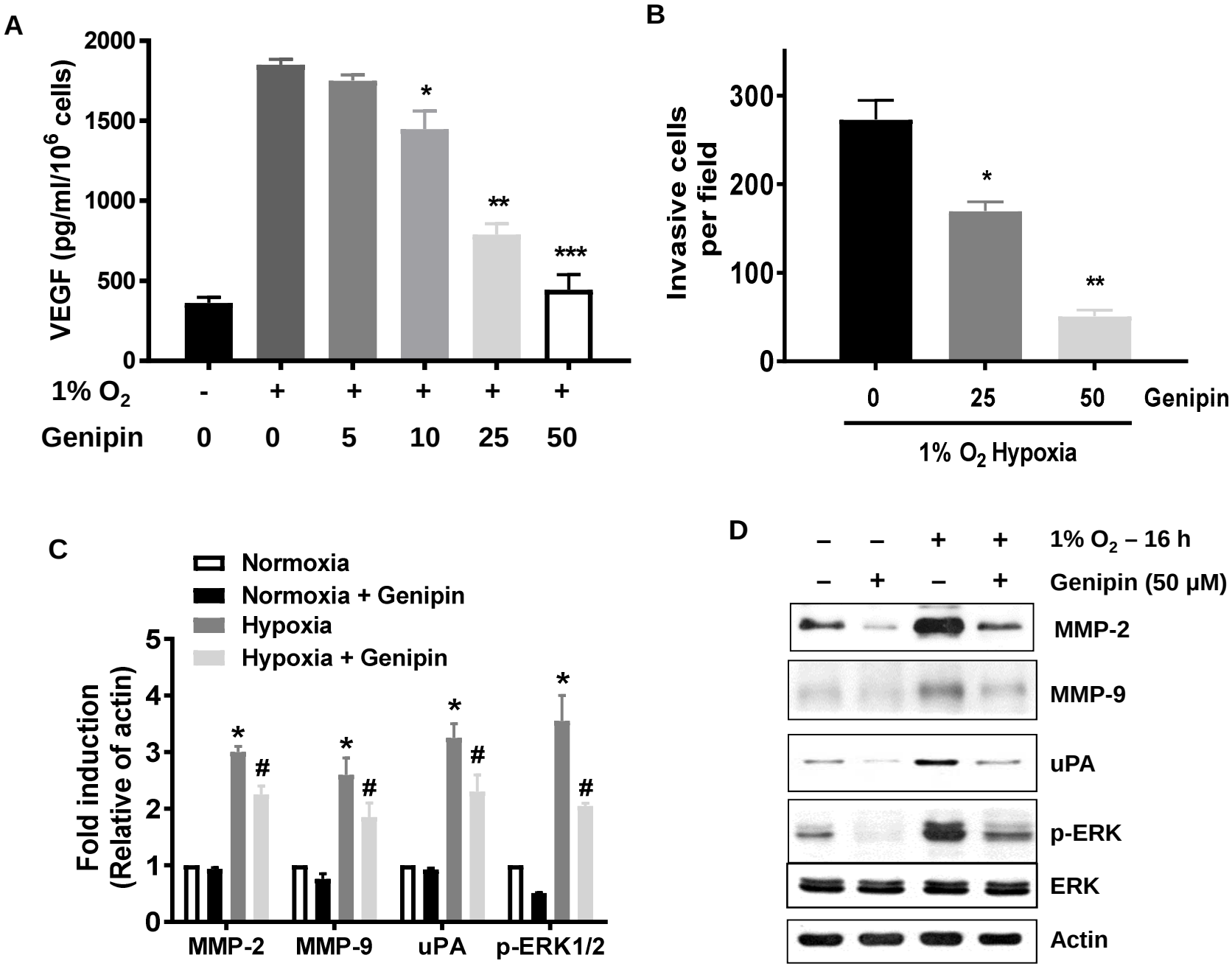

Fig. 5A, 5B, 5C, and 5D

Highlights

- Genipin inhibits accumulation of HIF-1α and VEGF expression in human colon cancer.
- Genipin exerts anti-invasion effect on human colon cancer cells.
- Genipin-induced anti-cancer effect is exerted by Akt and ERK signaling pathways.