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Flavonoids-Induced Accumulation of Hypoxia-Inducible Factor (HIF)-1α/2α Is Mediated Through Chelation of Iron

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Abstract Hypoxia-inducible factor-1 alpha (HIF-1α) is the regulatory subunit of the heterodimeric transcription factor HIF-1 that is the key regulator of cellular response to low oxygen tension. Under normoxic conditions, HIF-1α is continuously degraded by the ubiquitin-proteasome pathway through pVHL (von Hippel–Lindau tumor suppressor protein). Under hypoxic conditions, HIF-1α is stabilized and induces the transcription of HIF-1 target genes. Quercetin, a flavonoid with anti-oxidant, anti-inflammatory, and kinase modulating properties, has been found to induce HIF-1α accumulation and VEGF secretion in normoxia. In this study, the molecular mechanisms of quercetin-mediated HIF-1α accumulation were investigated. Previous studies have shown that, in addition to being induced by hypoxia, HIF-1α can be induced through the phosphatidylinositol 3-kinase (PI3K)/Akt and p53 signaling pathways. But our study revealed, through p53 mutant-type as well as p53 null cell lines, that neither the PI3K/Akt nor the p53 signaling pathway is required for quercetin-induced HIF-1α accumulation. And we observed that HIF-1α accumulated by quercetin is not ubiquitinated and the interaction of HIF-1α with pVHL is reduced, compared with HIF-1α accumulated by the proteasome inhibitor MG132. The use of quercetin's analogues showed that only quercetin and galangin induce HIF-1/2α accumulation and this effect is completely reversed by additional iron ions. This is because quercetin and galangin are able to chelate cellular iron ions that are cofactors of HIF-1/2α proline hydroxylase (PHD). These data suggest that quercetin inhibits the ubiquitination of HIF-1/2α in normoxia by hindering PHD through chelating iron ions. J. Cell. Biochem. 103: 1989–1998, 2008.

Key words: HIF-1α; quercetin; galangin; p53; $FeSO_4$

Hypoxia-inducible factor 1 (HIF-1) is heterodimeric transcription factor that plays a role in cellular response to low oxygen environment and contributes to angiogenesis, erythropoiesis, glucose metabolism, and cell proliferation [Guillemin and Krasnow, 1997; Semenza, 1999; Pugh and Ratcliffe, 2003]. The α-subunit is systematically regulated by oxygen level, whereas the β-subunit, known as aryl hydrocarbon receptor nuclear translocator, is constantly available [Maynard and Ohh, 2004]. In normoxic condition, HIF-1α is hydroxylated by prolyl hydroxylase (PHD) and then recognized by E3 ubiquitin ligase complex that is composed of elongin B and C, Cul2, Rbx1, and pVHL. Protein of von Hippel–Lindau tumor suppressor gene (pVHL) is an E3 ligase that attaches ubiquitin to HIF-1α and induces its degradation through the proteasome pathway [Lisztwan

Abbreviations used: HIF-1α, hypoxia-inducible factor-1 alpha; MAPK, mitogen-activated protein kinase; PAGE, polyacrylamide gel electrophoresis; PARP, poly(ADP-ribose) polymerase; PBS, phosphate-buffered saline; PHD, proline hydroxylase; PI3K, phosphatidylinositol 3-kinase; pVHL, von Hippel–Lindau tumor suppressor protein; SDS, sodium dodecyl sulfate; VEGF, vascular endothelial growth factor.

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et al., 1999; Ohh et al., 2000]. The hypoxic condition makes PHD inactive so that pVHL cannot recognize HIF-1α, which results in the accumulation of HIF-1α and its translocation into the nucleus and subsequent transcriptional activation [Ivan et al., 2001; Jaakkola et al., 2001]. HIF-2α, an isoform of HIF-1α, is also regulated in the same way but its expression is known to be specific to tissue and developmental state [Hu et al., 2006].

The intracellular level of HIF-1α can be increased in normoxic conditions by several hormones and growth factors such as insulin, estrogen, angiotensin II, and epidermal growth factor [Zhong et al., 2000; Treins et al., 2002; Kazi and Koos, 2007]. These non-hypoxic activations are linked to phosphatidylinositol 3-kinase (PI3K)/Akt or mitogen-activated protein kinase (MAPK) signal pathways through the binding of mediators to their specific membrane receptors [Berra et al., 2000; Minet et al., 2001]. The PI3K/Akt signal pathway is required for cap-dependent HIF-1α mRNA translation and for heat shock protein to stabilize HIF-1α, although the underlying mechanisms remain to be elucidated [Zhou et al., 2004]. MAPK signal pathways are implicated to phosphorylation of HIF-1α. By phosphorylating HIF-1α, p38 MAPK inhibits the interaction between HIF-1α and pVHL, and p42/p44 MAPK promotes nuclear accumulation and transcriptional activity of HIF-1α through blocking its CRM1-dependent nuclear export [Kwon et al., 2005; Mylonis et al., 2006].

Previously quercetin, a flavonoid, was reported to stabilize HIF-1α protein and induce vascular endothelial growth factor (VEGF) in endothelial cells in normoxic conditions [Wilson and Poellinger, 2002]. Several researchers have reported that quercetin hinders Akt via inhibiting PI3K [Matter et al., 1992; Kim and Lee, 2007]. These observations are contradicted to above non-hypoxic pathway by which HIF-1α is accumulated through PI3K/Akt activation. In this study, we hypothesized that the quercetin-induced increase in intracellular level of HIF-1α is mediated through p53 accumulation [Mertens-Talcott et al., 2005]. It is well known that the p53 protein is expressed at low level in normoxia due to its fast turn-over via ubiquitin-proteasome pathway, in which Mdm2 plays a role as E3 ubiquitin ligase [Zhou et al., 2002]. As Akt-mediated Mdm2 phosphorylation is essential to p53 degradation [Feng et al., 2004], it is possible that quercetin-induced Akt inhibition leads to p53 accumulation through dephosphorylation of Mdm2. Previous studies also suggest cross-talks between HIF-1α and p53, including the interaction of HIF-1α with Mdm2 and p53 with pVHL [An et al., 1998; Ravi et al., 2000; Schmid et al., 2004]. Elevation of intracellular p53 level may be responsible for accumulation of HIF-1α. Contrary to our hypothesis, our studies demonstrate that p53 is not involved in quercetin-induced HIF-1α accumulation. Our studies reveal that quercetin and galangin, but not chrysin and wogonin, are able to increase the intracellular level of HIF-1α by chelating cellular iron ions.

MATERIALS AND METHODS

Cell Culture

Human prostate adenocarcinoma LNCaP, DU-145, and PC-3 cell lines were obtained from the American Type Culture Collection (Manassas, VA). Human colon carcinoma HCT116 $p53^{+/+}$ and $p53^{-/-}$ cell lines were obtained from Dr. B. Vogelstein (Johns Hopkins University, Baltimore, MD). LNCaP and PC-3 or DU-145 cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA) or DMEM medium (Gibco BRL, Gaithersburg, MD), respectively, with 10% fetal bovine serum (Hyclone, Logan, UT) and 26 μM sodium bicarbonate for monolayer cell culture. HCT116 cell lines were cultured in McCoy's 5A medium (Gibco BRL) with 10% fetal bovine serum. Cells were cultured at 37°C humidified atmosphere with 5% CO_2 in air.

Chemical Treatment

Quercetin, galangin, chrysin, and ferrous sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). Wogonin was obtained from Wako Chemicals (Richmond, VA). Proteasome inhibitor MG132 was obtained from Calbiochem (San Diego, CA). A stock solution of ferrous sulfate was prepared in DDW and the others were prepared in DMSO. Prior to experiments, cells were grown to about 70–80% confluence in 60 mm dishes or 6-well plates.

Antibodies

Polyclonal anti-Akt, anti-phospho-Akt, anti-phospho-Mdm2, anti-p53, and anti-pVHL were purchased from Cell Signaling (Beverly, MA),

anti-HIF-2α from Novus Biologicals (Littleton, CO), and anti-Mdm2 from Santa Cruz (Santa Cruz, CA). Monoclonal antibodies were purchased from the following companies: anti-ubiquitin from Santa Cruz, and anti-HIF-1α from BD Biosciences (San Jose, CA).

Cell Lysates and SDS–PAGE

Cells were lysed with 1× Laemmli lysis buffer (2.4 M glycerol, 0.14 M Tris, pH 6.8, 0.21 M SDS, and 0.3 mM bromophenol blue) and boiled for 10 min. Protein concentrations were measured with BCA Protein Assay Reagent (Pierce, Rockford, IL). The samples were diluted with 1× lysis buffer containing 1.28 M β-mercaptoethanol, and equal amounts of protein were loaded on 8–12% SDS-polyacrylamide gels. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis was done according to Laemmli using a Hoefer gel apparatus.

Immunoblot Analysis

Proteins in gel were electrophoretically transferred to polyvinylidene difluoride membrane. The membranes were incubated with 5% (w/v) skim milk in phosphate-buffered saline solution (PBS) containing 0.1% Tween-20 for blocking and then reacted with primary antibody. Horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG was used as secondary antibody. Proteins were visualized by the chemiluminescence protocol (ECL, Amersham, Arlington Heights, IL).

VEGF Secretion Assay

Equal numbers of DU-145 cells were seeded to 35-mm Petri dishes and grown overnight, and treated with 100 μM quercetin. The supernatants were collected and stored at −70°C for a VEGF secretion assay. The amount of secreted VEGF was measured using a Quantikine human VEGF ELISA immunoassay (R&D Diagnostics, Minneapolis, MN).

RESULTS

Effect of Quercetin on the Mdm2-p53 Pathway

To confirm whether quercetin induces HIF-1α accumulation, human prostate adenocarcinoma LNCaP cells were treated with various concentrations (10–100 μM) of quercetin for 8 h. Figure 1A shows that the intracellular level of HIF-1α was increased by treatment with quercetin (50–100 μM). To examine whether the Akt-Mdm2-p53 pathway is responsible for quercetin-induced accumulation of HIF-1α in normoxic conditions, LNCaP cells, which contain wild-type p53, were treated with various concentrations of quercetin for 8 h. Figure 1B shows that quercetin dephosphorylated Akt in dose-dependent manner without changing the total level of Akt. As expected, quercetin also dephosphorylated Mdm2 and decreased the intracellular level of Mdm2. Unlike Mdm2, the intracellular level of p53 was significantly increased. To investigate whether p53 plays an important role in an increase in HIF-1α level

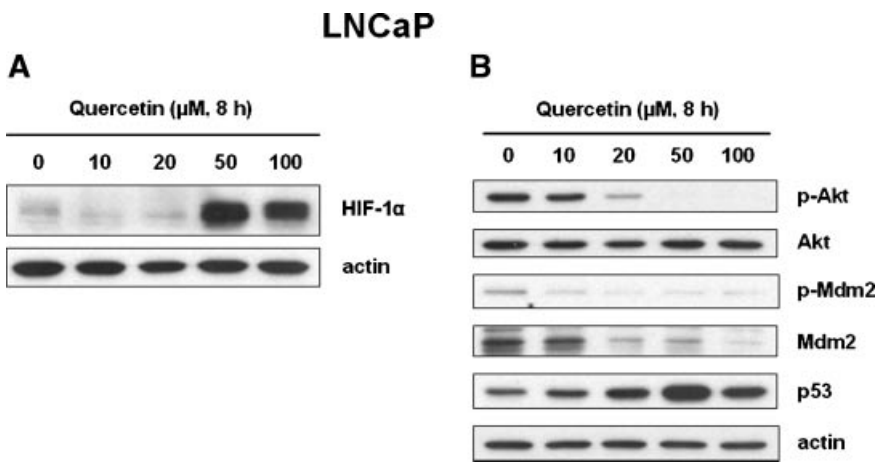

Fig. 1. Effects of quercetin on intracellular level of HIF-1α (**A**) and Akt-Mdm2-p53 pathway (**B**) in LNCaP cells. Cells were incubated with various concentrations (10–100 μM) of quercetin for 8 h then harvested. Cell lysates containing equal amounts of protein (20 μg) were separated by SDS–PAGE and immunoblotted with (A) anti-HIF-1α antibody, and (B) anti-phospho-Akt, anti-Akt, anti-phospho-Mdm2, anti-Mdm2, or anti-p53 antibody. Actin was used as a loading control.

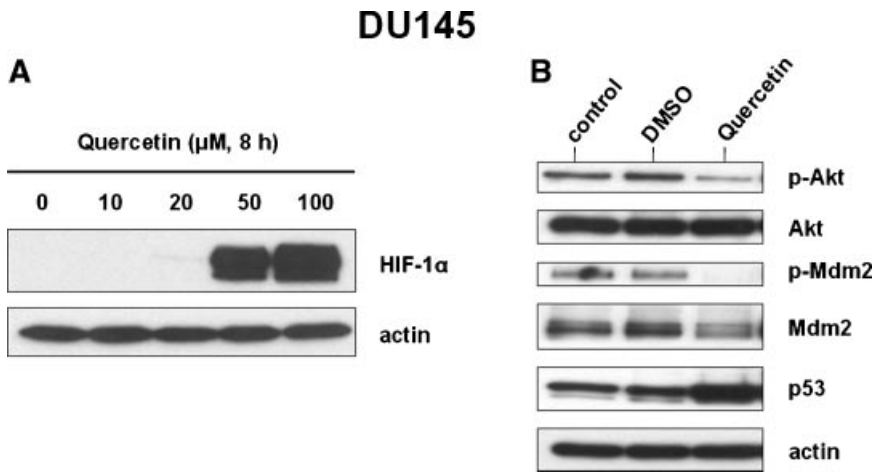

Fig. 2. Effects of quercetin on intracellular level of HIF-1α (**A**) and Akt-Mdm2-p53 pathway (**B**) in DU-145 cells. Cells were treated with various concentrations (10–100 μM) of quercetin for 8 h (A) or cells were treated with either 1% DMSO or 100 μM quercetin for 8 h (B). Cells were harvested and cell lysates containing equal amounts of protein (20 μg) were separated by SDS–PAGE and then immunoblotted with (A) anti-HIF-1α antibody, and (B) anti-phospho-Akt, anti-Akt, anti-phospho-Mdm2, anti-Mdm2, or anti-p53 antibody. Actin was used to confirm the amount of proteins loaded in each lane.

during quercetin treatment, DU-145 cells, which contain mutant-type p53, were treated with quercetin (10–100 μM) for 8 h. Like LNCaP cells, the intracellular level of HIF-1α was increased in the presence of quercetin (50–100 μM; Fig. 2A). Quercetin dephosphorylated Akt and Mdm2 while it increased the intracellular level of p53 (Fig. 2B). These results suggest that quercetin increases the intracellular level of HIF-1α regardless of p53 status.

Quercetin-Induced HIF-1α Accumulation Is Independent of p53 Status

To confirm our observations, three different cell lines (p53 wild-type cell LNCaP, p53 mutant-type cell DU-145, and p53 null cell PC-3) were employed and treated with 100 μM quercetin for various times (4–8 h). Figure 3A clearly demonstrates that the intracellular level of HIF-1α was increased regardless of p53 status. To clarify the p53 role in HIF-1α accumulation, isogenic human colon carcinoma cell lines (HCT116) differing only in the presence or absence of wild-type p53 were used. Figure 3B shows that quercetin increased the intracellular level of HIF-1α in both HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells. These results clearly suggest that quercetin-induced HIF-1α accumulation is independent of p53 status.

Quercetin Stimulates Secretion of VEGF

It is well known that HIF-1α controls the expression of VEGF [Towler, 2007]. It is possible that quercetin up-regulates VEGF through elevation of HIF-1α level. This possibility was examined by analyzing VEGF secretion level during treatment with quercetin in DU-145 cells. Figure 4A clearly revealed that quercetin

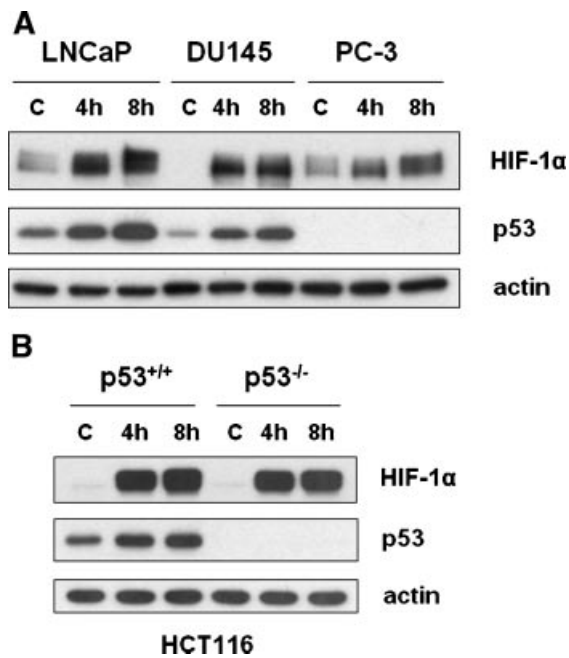

Fig. 3. Effect of quercetin on HIF-1α accumulation in various p53 statuses. **A**: LNCaP, DU-145, and PC-3 cells were treated with 100 μM quercetin for various times (4–8 h). **B**: HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells were treated with 100 μM quercetin for various times (4–8 h). Equal amounts of cell lysates (20 μg) were subjected to electrophoresis and analyzed by Western blot as described above.

increased the intracellular level of HIF-1α in time dependent manner. VEGF induction was also increased during treatment with quercetin (Fig. 4B).

Quercetin Inhibits HIF-1α Ubiquitination

In normoxic condition, HIF-1α is continuously expressed and simultaneously degraded through the ubiquitin-proteasome pathway. It is possible that quercetin-mediated accumulation of HIF-1α is due to inhibition of HIF-1α degradation. To test this possibility, the effect of quercetin on the HIF-1α degradation pathway was investigated in the presence or absence of proteasome inhibitor MG132. Intracellular level of HIF-1α was increased by treatment with quercetin or MG132. However, the HIF-1α increment pattern by quercetin was very different from that by MG132 (upper panels in Fig. 5). Unlike quercetin, MG132 induced an accumulation of ubiquitinated HIF-1α. Moreover, the accumulation of ubiquitinated forms in the sample was decreased by combined treatment with quercetin and MG132. These results suggest that the mechanism of HIF-1α accumulation by quercetin is not the same as that by MG132. This difference was further investigated by immunoprecipitation assay (lower panels in Fig. 5). Quercetin accumulated HIF-1α by inhibiting ubiquitination. Unlike quercetin, MG132 accumulated HIF-1α by inhibiting proteasome, but not by inhibiting ubiquitination. Quercetin inhibited interaction between HIF-1α and pVHL; in contrast, interaction between HIF-1α and pVHL was increased in the presence of MG132 and its effect was suppressed by adding quercetin. These results suggest that quercetin induces an accumulation of HIF-1α by inhibiting interaction between HIF-1α and pVHL rather than inhibiting proteasomes.

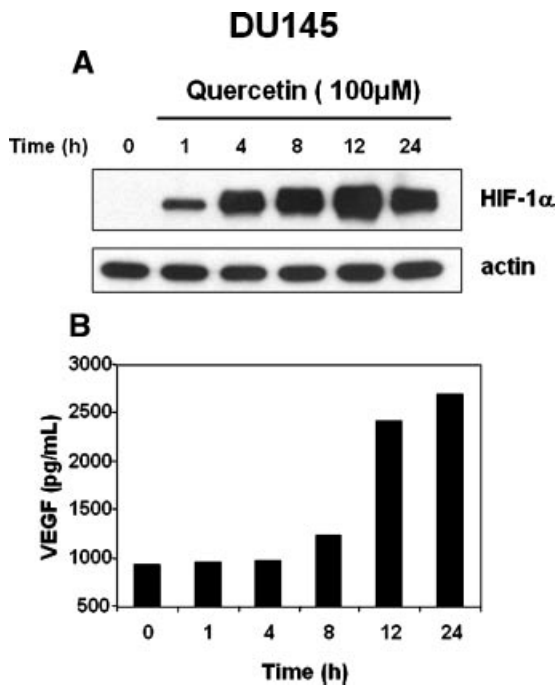

Fig. 4. Quercetin-induced HIF-1α accumulation and VEGF secretion. DU-145 cells were treated with 100 μM quercetin for various times (1–24 h). **A**: Cell lysates containing equal amounts of protein (20 μg) were separated by SDS–PAGE and then immunoblotted with anti-HIF-1α antibody. Actin was used as a loading control. **B**: Each supernatant of cell culture was used to measure the secreted VEGF.

Effect of Quercetin and Its Analogues on HIF-1α/2α Accumulation

Previous studies have shown that regulatory mechanisms of HIF-2α are similar to that of HIF-1α [Löfstedt et al., 2007]. To understand biochemical effects of quercetin on HIF-1α and HIF-2α, several quercetin analogues including galangin, chrysin, and wogonin were employed (Fig. 6A). Cells were treated with each flavonoid and its effect on HIF-1α as well as HIF-2α was investigated. As shown in Figure 6B, quercetin and galangin, but not chrysin and wogonin,

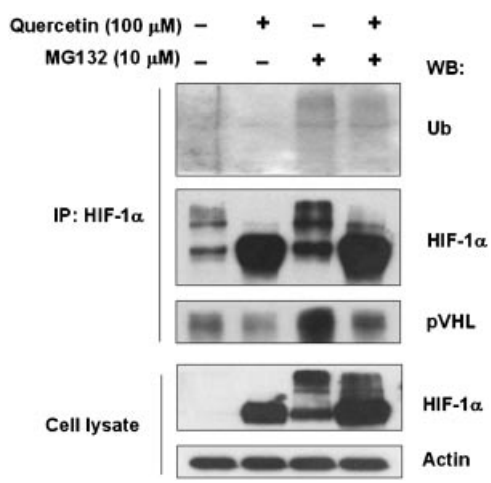

Fig. 5. Effect of quercetin on HIF-1α ubiquitination. DU-145 cells were treated for 8 h with 100 μM quercetin in the presence or absence of 10 μM MG132 and then harvested. Cell lysates were immunoprecipitated with anti-HIF-1α antibody and immunoblotted with anti-Ub, anti-HIF-1α, or anti-pVHL (**upper panels**). Equal amounts of protein (20 μg) were separated by SDS–PAGE and immunoblotted with anti-HIF-1α or anti-actin antibody (**lower panels**).

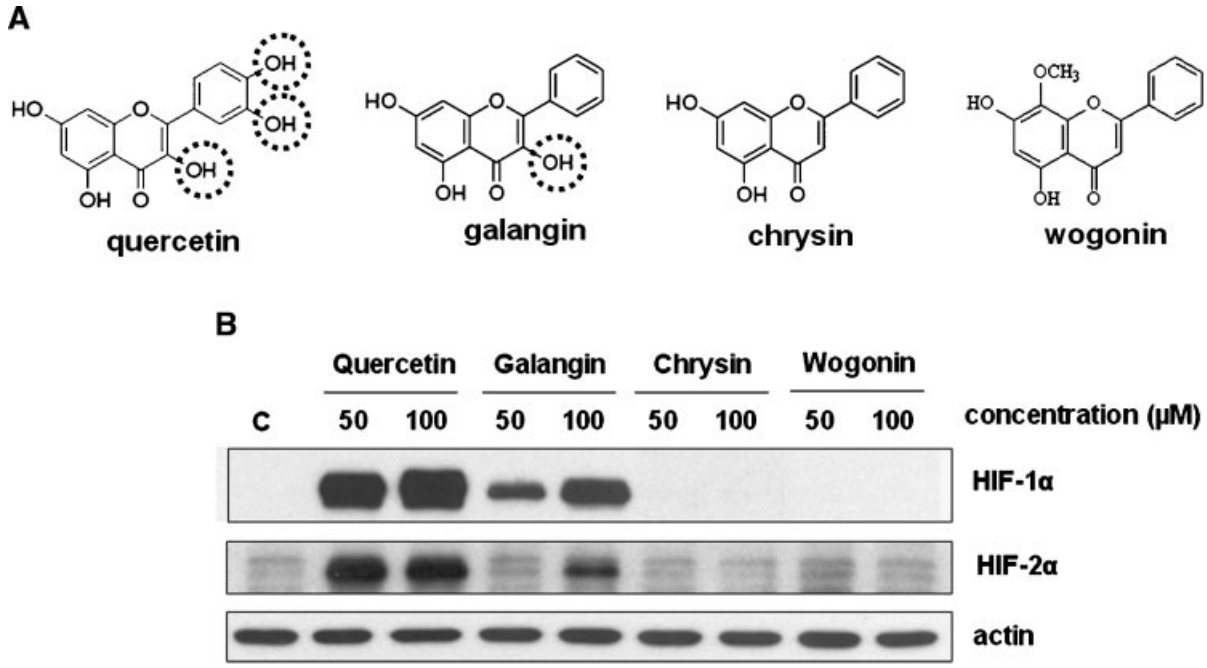

Fig. 6. Effect of quercetin and its analogues on HIF-1α/2α accumulation in DU-145 cells. **A**: Chemical structures of quercetin and its analogues, galangin, chrysin, and wogonin. **B**: Cells were treated for 8 h with various concentrations (50–100 μM) of quercetin, galangin, chrysin, or wogonin and then harvested. Equal amounts of protein (20 μg) were separated by SDS–PAGE and immunoblotted with anti-HIF-1α or anti-HIF-2α antibody. Actin was shown as an internal standard.

induce HIF-1α and HIF-2α accumulation. These results suggest that biochemical effects of quercetin and galangin on HIF-1α and HIF-2α are similar to each other. It is quite interesting to compare molecular structures of these four flavonoids. The structural difference of quercetin and galangin compared to chrysin and wogonin is a 3-hydroxy moiety. In particular, this is the only a difference between galangin and chrysin (Fig. 6A).

Iron Reverses HIF-1α/2α Accumulation by Flavonoids

Flavonoids are well known to function as antioxidants [Lamson and Brignall, 2000]. Of several reported antioxidant mechanisms, metal chelation has been extensively studied [Leopoldini et al., 2006]. Some flavonoids are able to chelate transition metal ions, resulting in stable complexes that prevent them from participating in free radicals generation [Engelmann et al., 2005]. It is well known that PHD plays an essential role in HIF-1α regulation. This enzyme requires Fe^{2+} ion as a cofactor. Recent studies have shown that quercetin is able to chelate iron ion with 1:1 and 1:2 iron-quercetin stoichiometries through 3′,4′-hydroxy, 3-hydroxy and 4-oxo, and 5-hydroxy and 4-oxo groups, respectively [Leopoldini et al., 2006]. We hypothesized that quercetin inhibits PHD by chelating Fe^{2+} ion in cells. To test the hypothesis, we examined whether quercetin-induced HIF-1α accumulation is reversible by the Fe^{2+} ion. After 4 h incubation of DU-145 cells with 100 μM quercetin, Fe^{2+} ions were added to the culture medium and the intracellular level of HIF-1α protein was examined using Western blot analysis (Fig. 7A). Figure 7A shows that the accumulated HIF-1α proteins were completely reduced within 1 h treatment with $FeSO_4$. The reduction of quercetin-induced HIF-1α accumulation was dependent upon $FeSO_4$ concentrations (Fig. 7B); this requires at least 20 μM $FeSO_4$ against 100 μM quercetin treatment. Figure 7C shows that not only quercetin but also galangin induced accumulation of HIF-1α in a variety of cell lines (DU-145, PC-3, and LNCaP), even though the amount of accumulation was somewhat dependent upon cell types. Both drugs also induced accumulation of HIF-2α in DU-145 and PC-3 cells, although there was no detectable level of HIF-2α in LNCaP cells. Nonetheless, $FeSO_4$ effectively inhibited accumulation of HIF-1α

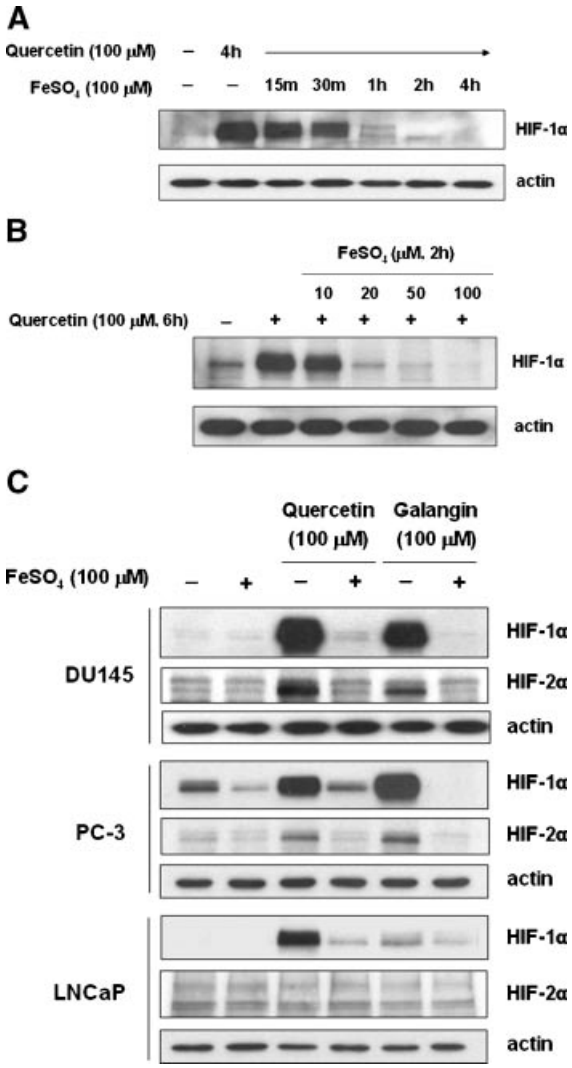

Fig. 7. Effect of iron on quercetin/galangin-induced HIF-1α/2α accumulation. **A**: DU-145 cells were treated with 100 μM quercetin for 4 h and then ferrous sulfate (100 μM) was added for various times (15 min to 4 h). **B**: DU-145 cells were treated for 8 h with 100 μM quercetin in the presence or absence of various concentrations (10–100 μM) of $FeSO_4$. **C**: DU-145, PC-3, and LNCaP cells were incubated for 8 h with 100 μM quercetin or galangin in the presence or absence of 100 μM ferrous sulfate. Equal amounts of protein (20 μg) were separated by SDS–PAGE and immunoblotted with anti-HIF-1α or anti-HIF-2α antibody. Actin was shown as an internal standard.

and HIF-2α. Based on our observations and the literature, we proposed a model in Figure 8. Quercetin and galangin-induced accumulation of HIF-1α is mediated through chelating iron ion in normoxic conditions. Chelation of the Fe^{2+} ion, which is a cofactor for the PHD enzyme, leads to inhibition of PHD and subsequently inhibits ubiquitination of HIF-1α/2α.

DISCUSSION

Previous studies have demonstrated that the PI(3)K-Akt-Mdm2 pathway regulates HIF-1α expression and HIF-1 activity under normoxic conditions [Bárdos et al., 2004]. HIF-1α directly interacts with Mdm2 and significantly suppresses Mdm2-mediated p53 ubiquitination and blocks Mdm2-mediated nuclear export of p53 [Chen et al., 2003]. On the other hand, an increase in Mdm2 results in decreased levels of p53 followed by increased expression of HIF-1α [Kwon et al., 2004; Nieminen et al., 2005]. However, our studies reveal p53-independent accumulation of HIF-1α under normoxic conditions by treatment with quercetin.

A decrease in cellular oxygen level leads to HIF-1α activation via stabilization of the HIF-1α protein and conversely proteolysis of HIF-1α through the ubiquitin-proteasome pathway in response to reoxygenation results in rapid decrease of HIF-1α activity. The key factor in this delicate HIF-1α regulation is oxygen-sensing by PHD that hydroxylates proline residues at positions 402 and 564 using cellular oxygen [Ivan et al., 2001]. Subsequently, pVHL binds to the hydroxylated HIF-1α and marks it for proteasomal degradation by ubiquitination [Ohh et al., 2000]. PHD requires oxygen and also iron ion and its enzymatic function is hindered by treatments such as desferrioxamine (DFX), cobalt chloride, and hypoxia. While hypoxic condition makes oxygen unavailable, DFX and cobalt chloride affect on iron ions by chelating and substituting, respectively, effects which are well known to stimulate the transcription of several genes that are associated with hypoxia [Guillemin and Krasnow, 1997].

The major observation reported here is that HIF-1α/2α accumulation by quercetin or galangin under normoxic conditions is quite similar to accumulation by DFX and cobalt chloride, which is induced by making iron ions unavailable to PHD. These results were consistent with previous observations [Wilson and Poellinger, 2002; Jeon et al., 2007; Triantafyllou et al., 2007]. One iron- and two iron-chelating moieties in galangin and quercetin, OH at position 3 of the C ring and/or OH at positions 3′ and 4′ of the B ring, enabled these flavonoids to inhibit PHD and subsequently inhibit ubiquitination of HIF-1α (Fig. 6). Quercetin which contains two iron-chelating moieties induces HIF-1α accumulation more effectively than galangin which

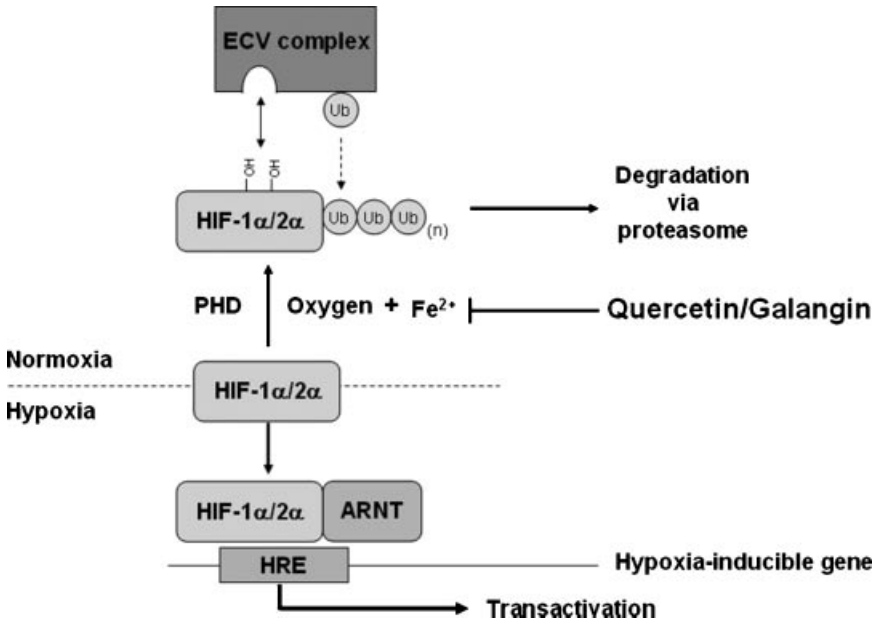

Fig. 8. A model for HIF-1α accumulation by quercetin/galangin in normoxic conditions.

contains one iron-chelating moiety (Fig. 6) [Kim et al., 2006]. Unlike quercetin and galangin, chrysin and wogonin do not contain an iron-chelating moiety and do not induce HIF-1α accumulation (Fig. 6). Intracellular accumulation of HIF-1α subsequently increases secretion of VEGF (Fig. 4) [Jeon et al., 2007].

Three isoforms of the α subunit of HIF-1 have been identified: HIF-1α, -2α (also referred to as EPAS-1, MOP2, HLF, and HRF) and -3α. These three isoforms compete for binding with HIF-1β and form HIF-1 [Wang et al., 1995], HIF-2 [Sowter et al., 2003], and HIF-3 [Maynard et al., 2003] complexes. Initial studies showed that the levels and activity of both HIF-1α and HIF-2α are regulated by PHD and the factor inhibiting HIF (FIH) in an O_2-, iron-, and 2-oxoglutarate (2OG)-dependent manner, giving them regulation of both protein stability and p300/CBP recruitment [Schofield and Ratcliffe, 2004]. HIF-2 regulates a set of targets partially overlapping with those of HIF-1 [Raval et al., 2005]. However, recent studies with *Hif-1α*$^{-/-}$ *and Hif-2α*$^{-/-}$ embryonic stem (ES) cells demonstrated differential regulation of the transcriptional activities of HIF-1α and HIF-2α. Our results revealed that quercetin and galangin induce HIF-1α accumulation in various types of prostate cancer cells (Figs. 1–3). Interestingly, LNCaP cells which are androgen-dependent, poorly invasive and tumorigenic contain little or no HIF-2α (Fig. 7C). Unlike LNCaP, DU-145, and PC-3 cells which are androgen-independent and tumorigenic contain HIF-2α (Fig. 7C). These observations were consistent with previous reports that HIF-2α has more profound effects on tumorigenesis and tumor growth [Covello et al., 2005, 2006; Hu et al., 2006]. Unlike HIF-1α and HIF-2α, HIF-3 was reported to be transcriptionally inactive and may possibly act as a dominant negative inhibitor [Jelkmann, 2004].

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