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Biochemical and Biophysical Research Communications 334 (2005) 543–548
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Epigallocatechin gallate inhibits HIF-1α degradation in prostate cancer cells

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Received 3 June 2005
Available online 1 July 2005

Abstract

Hypoxia-inducible factor-1, an αβ heterodimeric transcription factor, consists of a constitutively expressed HIF-1β subunit and a hypoxia-inducible HIF-1α subunit, and contributes to hypoxia-mediated tumor angiogenesis. Numerous epidemiologic and laboratory studies indicate that green tea has cancer preventive activity which has been attributed to its polyphenol components, the major one being epigallocatechin gallate (EGCG). This study investigated the effect of EGCG on normoxic HIF-1α expression in human prostate cancer cells. Surprisingly, we observed an EGCG-induced-dose-dependent increase in HIF-1-mediated transcription and HIF-1α protein levels under normoxia. However, concomitant treatment of the prostate cancer cells with EGCG and ferrous ions abolished the increase in HIF-1-mediated transcription that was seen with EGCG treatment alone, suggesting that EGCG may act as a ferrous ion chelator. Furthermore, we determined, for the first time, that EGCG inhibits prolyl hydroxylation of HIF-1α, thus preventing HIF-1α and pVHL interaction.

Keywords: Green tea; Epigallocatechin gallate; Prostate cancer; Hypoxia-inducible factor-1

During hypoxia, hypoxia-inducible factor-1 (HIF-1) activates transcription of a vast array of hypoxia-responsive genes, including those involved in angiogenesis, erythropoiesis, glucose metabolism, cell survival, and tissue invasion [1]. HIF-1, an αβ heterodimeric transcription factor, consists of a constitutively expressed HIF-1β subunit, and a hypoxia-inducible HIF-1α subunit. Under normoxic conditions, HIF-1α is hydroxylated at proline residues 402 and 564 within an oxygen-dependent degradation domain (ODD) [2–5], by Fe^{2+}-dependent HIF-1 prolyl hydroxylases (HPH) that use O_2 as a substrate [2,6]. Because O_2 appears to be rate limiting for prolyl hydroxylase activity, these enzymes may represent cellular O_2 sensors [2,7,8]. The von Hippel–Lindau protein (pVHL), the substrate recognizing component of an E3 ubiquitin ligase complex, links hydroxylated HIF-1α to the ubiquitination machinery and proteasome-mediated degradation [9–11]. This mechanism ensures very low cellular HIF-1α levels under normoxia. Under hypoxic conditions, the activity of HPH is inhibited due to lack of O_2, thus allowing accumulation of HIF-1α which then translocates to the nucleus and associates with HIF-1β subunit [12]. The HIF-1αβ heterodimer binds to hypoxia response element (HRE) (5′-CGTG-3′) found in the promoter/enhancer regions of HIF-1 target genes, thereby activating their expression [8]. As HIF-1 is at the center of most adaptation responses of cancer cells to hypoxia, overexpression of HIF-1α has been associated with increased patient mortality in several cancer types [1].

Epigallocatechin gallate (EGCG) is the major epicatechin component in green tea leaves and has recently been studied intensively as an anti-carcinogenic and anti-angiogenic agent [13,14]. Several groups have shown the cancer-preventive and anti-angiogenic effects

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.06.114

of tea polyphenols on various cancers including skin, lung, pancreas, breast, and prostate Recently, Nam et al. [15] reported that EGCG (10–40 μM = 4.6–18.4 μg/ml) inhibited proteasomal activity in intact Jurkat leukemic cells and prostate cancer cells (PC-3 and LNCaP). In addition, most flavonoids possess metal chelating property due to the presence of a catechol structure [16]. They can bind and decrease the level of cellular ferric and ferrous ions. Thus, these data imply a potential regulation of HIF-1α protein by EGCG, since HIF-1α protein is hydroxylated by Fe^{2+}-dependent HPH and rapidly degraded by proteasomes under normoxia [17].

The PC-3ML prostate carcinoma cell line, a subline of the PC-3 cell line, has been characterized as a cell line with a highly invasive and bone-targeting metastatic phenotype [18]. This study investigated the effect of EGCG on HIF-1α transcription factor expression in normoxic prostate cancer cells.

Materials and methods

Materials. (−)-Epigallocatechin gallate (EGCG), ferrous sulfate ($FeSO_4$), desferrioxamine (DFX), and protease inhibitor cocktail were obtained from Sigma. 2-Oxoglutarate (2-OG) was obtained from Fisher Scientific. Primary antibodies to HIF-1α and HIF-1β were from Santa Cruz Biotechnology and anti-β-actin antibody was from Sigma. Secondary antibodies, horseradish peroxidase-conjugated anti-mouse IgG and anti-rabbit IgG, M-PER mammalian protein extraction reagent, and neutravidin beads were from Pierce. Dual luciferase reporter assay system, BL21 (DE3) pLysS competent cells, isopropyl-β-D-thiogalactopyranoside (IPTG), MagneHis protein purification system, TNT T7 Coupled Reticulocyte Lysate System, and RNase inhibitor were from Promega. PD-10 columns and L-[^{35}S]methionine were from Amersham Biosciences. Biotinylated test and positive control peptides were synthesized by Alpha Diagnostic International. Their sequences correspond to the oxygen-dependent degradation domain (ODD) of HIF-1α and are as follows: biotin-Acp-DLDLEMLAP*YIPMDDD FQL-COOH. P* represents Pro^{564}, the hydroxylation site for HPH, and this amino acid residue is hydroxylated in the positive control peptide.

Plasmid constructs. pGL3-6xHRE-Luc was a generous gift from Dr. Peter RatCliffe (University of Oxford) [17], and contained six copies of hypoxia response element (HRE) from the erythropoietin gene promoter linked to thymidine kinase basal promoter and firefly luciferase gene. pRL-TK (Promega) was used as transfection efficiency control and contained the thymidine kinase promoter linked to *Renilla* luciferase gene. pVHL in pcDNA3.1-V5 His and HPH-2C in pHIS-parallell were generous gifts from Dr. Richard K. Bruick (University of Texas, Southwestern Medical Center, Dallas, TX).

Cell culture. The PC-3ML prostate carcinoma cell line, a subline of the PC-3 cell line, was a generous gift from Dr. Mark Stearns (MCP-Hahnmann University, Philadelphia) [18]. PC-3 and PC-3ML cells were maintained in F-12K Nutrient Mixture (Kaighn's Modification) (Invitrogen/Gibco), supplemented with 10% fetal bovine serum (FBS), 100 μg/ml streptomycin sulfate, and 100 U/ml penicillin G sodium in a humidified 5% CO_2 incubator at 37 °C and routinely passaged when 80% to 90% confluent.

Transient transfections and reporter gene assay. PC-3 and PC-3ML cells were grown in 48-well plates in F-12K medium without antibiotics until 90% confluent. PC-3ML cells were then transiently transfected with pGL3-6xHRE-Luc test plasmid along with pRL-TK using Lipofectamine 2000 (Invitrogen). After 4 h post-transfection, medium was replaced with fresh F-12K serum free medium with or without EGCG. PC-3ML cells were harvested 24 h later for dual Luciferase assays (Promega) to determine HRE-mediated transcriptional activity. The firefly luciferase expression from pGL3-6xHRE-Luc and *Renilla* luciferase from pRL-TK were measured sequentially from a single sample in a TD-20/20 Luminometer (Turner Designs) according to the Dual-Luciferase Reporter System protocol (Promega). The activity of pGL3-6xHRE-Luc was normalized to the activity of the pRL-TK internal control to minimize experimental variability caused by differences in cell viability or transfection efficiency, and then represented as relative luciferase activity on a bar graph.

Whole cell lysate preparation. PC-3ML cells were harvested by scraping and washed in ice-cold PBS and lysed in M-PER reagent (Pierce Chemical, Rockford, IL) at 100 μl/10^6 cells. After 15 min incubation on ice, lysates were cleared by centrifugation at 10,000 rpm, at 4 °C. The resulting lysates were stored at −80 °C until needed. Protein concentration was measured by bicinchoninic acid (BCA) assay (Sigma).

Immunoblot analysis. Whole cell lysates (25–50 μg protein) were separated on a 4–20% gradient SDS–polyacrylamide gel (Cambrex) under reducing conditions and then transferred to a nitrocellulose membrane. The membrane was blocked for 1 h at room temperature in T-PBS, pH 7.5 (PBS with 0.2% Tween 20) with 5% non-fat dried milk. After washing, the blot was incubated with primary antibody for 1 h at RT. The blot was washed three times in T-PBS and incubated with HRP-coupled secondary antibody (Pierce Chemical, Rockford, IL) for 1 h at RT. After extensive washing, the bands were detected using SuperSignal West Femto Maximum Sensitivity Substrate System (Pierce). The resulting chemiluminescence was visualized by Chemi-Imager 4400 equipped with a ChemiNova CCD camera (Alpha Innotech, San Leandro, CA). Densitometric analysis of protein bands was performed using AlphaEase Software (Alpha Innotech). Biotinylated protein standard (Bio-Rad) and streptavidin–HRP conjugates were used to estimate protein MW on the blot. After stripping, the blot was reprobed with β-actin antibody to determine the equal loading of proteins. HIF-1α expression was normalized to β-actin density from the same sample and expressed as a fold-change, with HIF-1α in control PC-3ML cells set at 1.

In vitro HPH activity assay. His-tagged HPH-2C (His-HPH-2C) was expressed by transforming BL21 (DE3) pLysS cells with HPH-2 cDNA in pHIS-parallell vector. Cells expressing His-HPH-2C were induced with 1 mM IPTG overnight at 20 °C. The induced cells were lysed, and His-HPH-2C was purified using the MagneHis protein purification system (Promega) according to the protocol provided by the manufacturer. ^{35}S-labeled pVHL (^{35}S pVHL) was produced by carrying out an in vitro transcription translation (IVTT) reaction with TNT T7 Coupled Reticulocyte Lysate System (Promega) using pcDNA3.1-V5His-VHL plasmid in the presence of L-[^{35}S]methionine according to the manufacturer's instructions. The IVTT reaction (supplemented with protease inhibitor cocktail) was desalted using PD-10 desalting columns (Amersham Biosciences). Briefly, the PD10 column was equilibrated with 25 ml ice-cold buffer (20 mmol/L Tris–Cl, pH 7.5, 100 mmol/L NaCl, and 1 mmol/L EDTA). The IVTT reaction was then loaded onto the column and eluted with 15 ml of the same buffer. Fractions (0.5 ml) were collected and scintillation counted, and the peak pVHL fraction was used in the HPH activity assay.

For the HPH activity assay, 20 μl neutravidin beads were incubated with 10 μg biotinylated HIF-1α ODD (test peptide) or biotinylated-Pro^{564} hydroxylated HIF-1α ODD (positive control peptide) in 25 mmol/L Tris–Cl, pH 7.5 at room temperature for 1 h. Beads were washed three times with 1× Reaction buffer (10 mmol/L Tris–Cl, pH 7.5, 2.5 mmol/L KCl, 0.75 mmol/L $MgCl_2$, 1 mmol/L ascorbate, 1 mmol/L of 2-oxoglutarate, and 0.5 mmol/L DTT) to remove unbound peptides. To each reaction, 25 μl of 2× reaction buffer containing 10 μmol/L Fe^{2+}, EGCG (10–40 μg/ml), and 0.5 μg His-HPH-2C were added to the peptide-bound beads and incubated at room temperature for 1 h. The beads were washed three times with

NETN buffer (20 mmol/L Tris–Cl, pH 8.0, 100 mmol/L NaCl, 1 mmol/L EDTA, and 0.5% NP-40) and then incubated in 500 μl EBC buffer (50 mmol/L Tris–Cl, pH 8.0, 120 mmol/L NaCl, 0.5% NP-40), 5 μl ^{35}S pVHL, and EGCG (10–40 μg/ml) for 20 min on ice. The beads were washed three times with NETN buffer, and the radioactivity of the ^{35}S pVHL bound to the biotinylated peptides was quantified by liquid scintillation counting in a Beckman 6000IC liquid scintillation counter.

Results

EGCG upregulates HRE-mediated transcription under normoxia

To determine the effect of EGCG on HIF-1 transcription factor, we used reporter gene assay in which PC-3 and PC-3ML cells were transiently co-transfected with pGL3-6xHRE-Luc to test HIF-1 transcriptional activity, and pRL-TK as a control. PC-3 and PC-3ML cells were treated with EGCG at 0, 20, or 40 μg/ml for 24 h, followed by dual luciferase assay. As shown in Figs. 1A and B, EGCG significantly and specifically increased HRE-mediated luciferase activity from pGL3-6xHRE-Luc in a dose-dependent manner in PC-3 (Fig. 1A) and PC-3ML (Fig. 1B) cells, while luciferase activity from pRL-TK remained relatively unchanged. Overall, EGCG increased HRE-mediated promoter activity by approximately 4- and 10-fold at 20 and 40 μg/ml concentration (Fig. 1C), respectively, both in PC-3 and PC-3ML cells.

EGCG increases HIF-1α protein levels

In a recent study, EGCG has been shown to inhibit proteasome activity in PC-3 cells [15]. Thus, we hypothesized that EGCG might prevent the degradation of HIF-1α protein, leading to the accumulation of HIF-1α protein and increased transcriptional activity of HIF-1. To test this hypothesis, we examined HIF-1α protein regulation following EGCG treatment. PC-3ML cells were treated with 20 μg/ml EGCG, and HIF-1α and HIF-1β expression was determined by immunoblot. As shown in Fig. 2, EGCG treatment upregulated HIF-1α expression, but not the HIF-1β expression whose expression is constitutive. In dose–response experiments, EGCG increased HIF-1α protein levels in a dose-dependent manner (Fig. 3).

Fe^{2+} abolishes EGCG-induced increase in HRE-mediated transcription

Since HIF-1 prolyl hydroxylase (HPH) requires Fe^{2+} for its catalytic activity, we tested the effect of exogenous Fe^{2+} on the EGCG-mediated increase in promoter activity of pGL3-6xHRE-Luc in PC-3ML cells. PC-3ML cells were transiently co-transfected with pGL3-6xHRE-Luc and pRL-TK plasmids and treated with increasing concentration of EGCG in the absence or presence of 300 μmol/L $FeSO_4$ (Fig. 4). The luciferase activity from pGL3-6xHRE-Luc in EGCG-treated cells (40 μg/ml) was increased 2.7-fold compared to that of control cells. However, the addition of exogenous $FeSO_4$ abolished the EGCG-mediated increase in HRE-promoter activity. $FeSO_4$ by itself had no significant effect on HRE-promoter activity. Desferrioxamine (DFX), a known iron chelator, increased

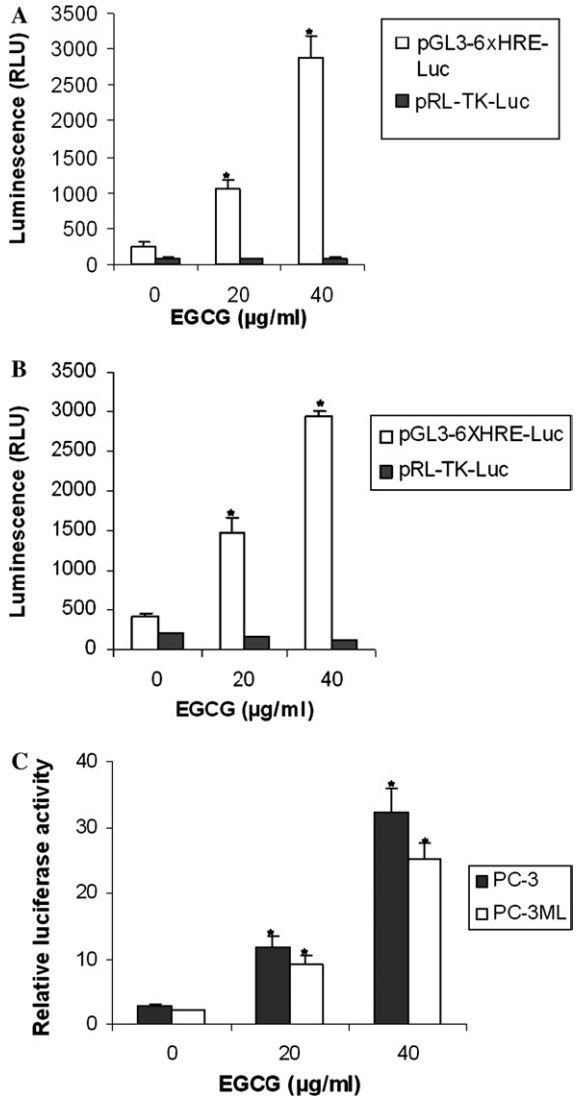

Fig. 1. EGCG upregulates HRE-mediated transcription under normoxia. PC-3 (A) and PC-3ML (B) cells were transiently co-transfected with pGL3-6xHRE-Luc and pRL-TK plasmids, and then treated with EGCG (0–40 μg/ml) in F12K SF for 24 h, followed by dual luciferase assay 24 h later. Reporter Luminescence from pGL3-6xHRE-Luc (□) and from pRL-TK (■) was measured and the value (RLU, Reporter Luminescence Unit) was plotted on a bar graph. (C) Firefly luciferase activity of pGL3-6xHRE-Luc was normalized to that of the pRL-TK internal control and was plotted as relative luciferase activity on a bar graph. Data are the means ± SD ($n = 3$). *$P < 0.05$ versus EGCG 0 μg/ml.

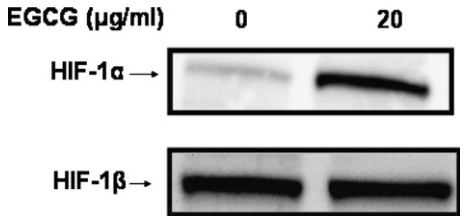

Fig. 2. EGCG increases HIF-1α protein levels. PC-3ML cells were treated with or without 20 μg/ml EGCG in F-12K SF for 24 h. Cell lysates were prepared and analyzed for HIF-1α and HIF-β expression by Western blot analysis as described in Materials and methods.

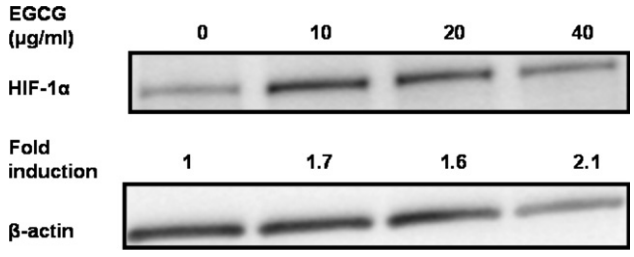

Fig. 3. Dose-dependent HIF-1α accumulation after EGCG treatment. PC-3ML cells were treated with 0–40 μg/ml of EGCG in F-12K SF, for 24 h. Whole cell lysates were prepared and analyzed for HIF-1α expression by immunoblot analysis. HIF-1α expression in each sample was normalized to β-actin density from the same sample and expressed as fold-change, with HIF-1α in the untreated PC-3ML cells set at 1.

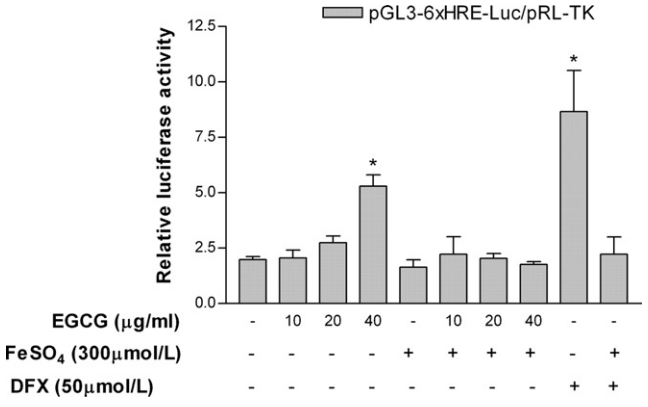

Fig. 4. Fe^{2+} abolishes EGCG-induced increase in HRE-mediated transcription. PC-3ML cells were transiently co-transfected with pGL3-6xHRE-Luc and pRL-TK plasmids and treated with none, $FeSO_4$ alone, EGCG (0–40 μg/ml) ±300 μmol/L $FeSO_4$, or DFX (100 μmol/L) ± 300 μmol/L $FeSO_4$ in F-12K SF for 24 h. Firefly luciferase activity of pGL3-6xHRE-Luc was normalized to that of the pRL-TK internal control and was plotted as relative luciferase activity on a bar graph. Data are the means ± SD ($n = 3$). *$P < 0.05$ versus EGCG 0 μg/ml.

HRE-mediated transcription by 4.4-fold, and this increase was abolished by the addition of $FeSO_4$. Thus, these results strongly suggest that the metal ion chelating activity of EGCG played an important role in increasing HRE-mediated transcription.

EGCG inhibits HPH activity

HPH requires Fe^{2+} for its activity to hydroxylate HIF-1α. Thus, we hypothesized that EGCG inhibits HPH activity and consequently inhibits HIF-1α ubiquitination and degradation. HPH-mediated hydroxylation of Pro^{564} within the ODD of HIF-1α is required for the interaction of HIF-1α with pVHL. To test our hypothesis that EGCG inhibits HPH activity, in vitro HPH activity assays were performed. In this assay, HIF-1α ODD peptides bound to neutravidin beads via their biotin moieties were hydroxylated at Pro^{564} by recombinant HPH-2C. The prolyl-hydroxylated HIF-1α ODD peptide would then bind to ^{35}S pVHL. Thus, the radioactivity measured in this assay was directly related to the amount of ^{35}S pVHL bound to the HIF-1α ODD peptide and the hydroxylation status of HIF-1α ODD peptide. As shown in Fig. 5A, the binding of ^{35}S pVHL to HIF-1α ODD peptide was dependent on the presence of recombinant HPH-2C and thus the hydroxylation of HIF-1α ODD peptide. However, EGCG treatment

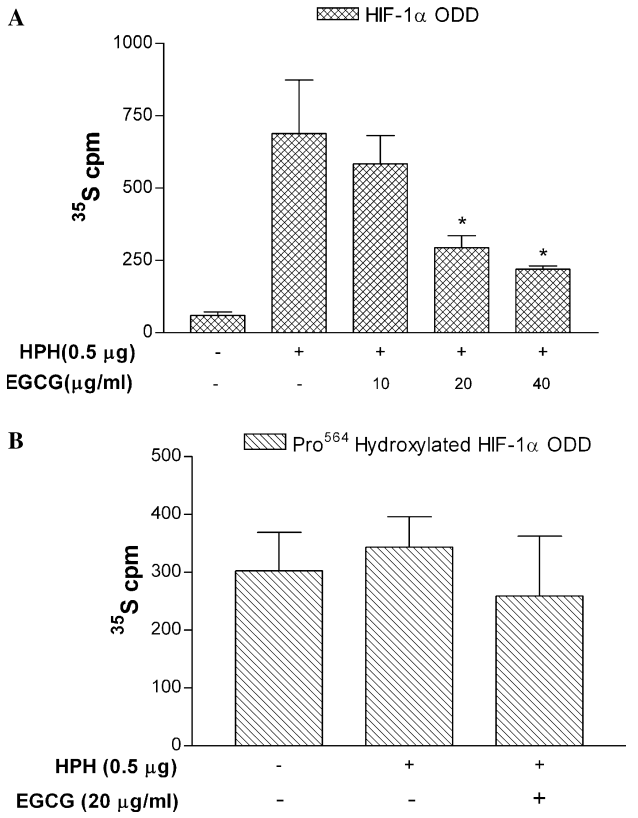

Fig. 5. EGCG inhibits in vitro HPH activity. Synthetic biotinylated peptides corresponding to (A) HIF-1α C-terminal ODD (556–574) or (B) Pro^{564} hydroxylated HIF-1α C-terminal ODD (556–574) were bound to neutravidin beads and incubated with recombinant HPH-2C for 1 h in the absence or presence of EGCG (10–40 μg/ml), followed by a 20-min incubation with ^{35}S pVHL. ^{35}S pVHL bound to the peptide was measured by scintillation counting. Data are the means ± SD ($n = 2$). *$P < 0.05$ versus reaction containing 0.5 μg HPH and no EGCG.

decreased the ^{35}S pVHL capture in a dose-dependent manner, with 40 μg/ml EGCG lowering the ^{35}S pVHL capture by almost 70%. On the other hand, EGCG had no significant effect on the interaction between ^{35}S pVHL and the positive control peptide containing HIF-1α ODD hydroxylated at Pro^{564} (Fig. 5B), indicating that EGCG did not exert any direct inhibitory effect on pVHL and HIF-1α ODD interaction. Thus, these data strongly support the hypothesis that EGCG has the ability to directly inhibit HIF-1α hydroxylation, thus preventing pVHL-HIF-1α ODD interaction.

Discussion

Herein, we determined that EGCG upregulated HRE-mediated promoter activity in hormone-refractory prostate cancer cells, PC-3 and PC-3ML cells. Since pGL3-6xHRE-Luc and pRL-TK plasmids have the same thymidine kinase basal promoter, this result suggested that EGCG specifically upregulated HRE-mediated promoter activity without affecting basal transcriptional activity. Furthermore, this study provides the first evidence of EGCG, a major green tea polyphenol, inhibiting HIF-1α hydroxylation and subsequent HIF-1α degradation in normoxia. The evidence that most tea polyphenols including EGCG are known to be metal ion chelators [16], and that HPH requires Fe^{2+} for its catalytic activity led us to hypothesize that EGCG might inhibit HPH activity. The data from Fig. 5 strongly supported the role of EGCG as a HPH activity inhibitor. Inhibition of pVHL and HIF-1α ODD peptide interaction indicated inhibition of prolyl hydroxylation by HPH in the presence of EGCG since prolyl hydroxylation was a prerequisite to HIF-1α ODD and pVHL interaction. EGCG inhibited HIF-1α prolyl hydroxylation and the subsequent pVHL interaction, leading to HIF-1α protein stabilization and subsequent increase in HIF-1α transcriptional activity. A recent study by Zhou et al. [19] showed induction of HIF-1 activity by EGCG and epicatechin gallate (ECG) in T47D breast cancer cells, in which ECG had a greater HIF-1 inducing effect.

Based on our results, we propose a working model of EGCG's mechanism of action in increasing HIF-1 transcriptional activity. As shown in Fig. 6, EGCG prevents HIF-1α hydroxylation by inhibiting HPH activity. This leads to inhibition of HIF-1α–pVHL interaction, blocking HIF-1α ubiquitination and proteasomal degradation. Then, HIF-1α subunits dimerize with HIF-1β subunits and induce HIF-1-responsive genes. EGCG had no direct effect on HIF-1α-pVHL interaction as it did not inhibit binding of pVHL to HIF-1α ODD peptide containing hydroxylated Pro^{564} (positive control peptide). In addition to prolyl hydroxylation within the HIF-1α ODD, Asn^{803} in HIF-1α transactivation domain is hydroxylated by factor inhibiting HIF-1 (FIH-1) which also requires Fe^{2+} as a cofactor [20,21].

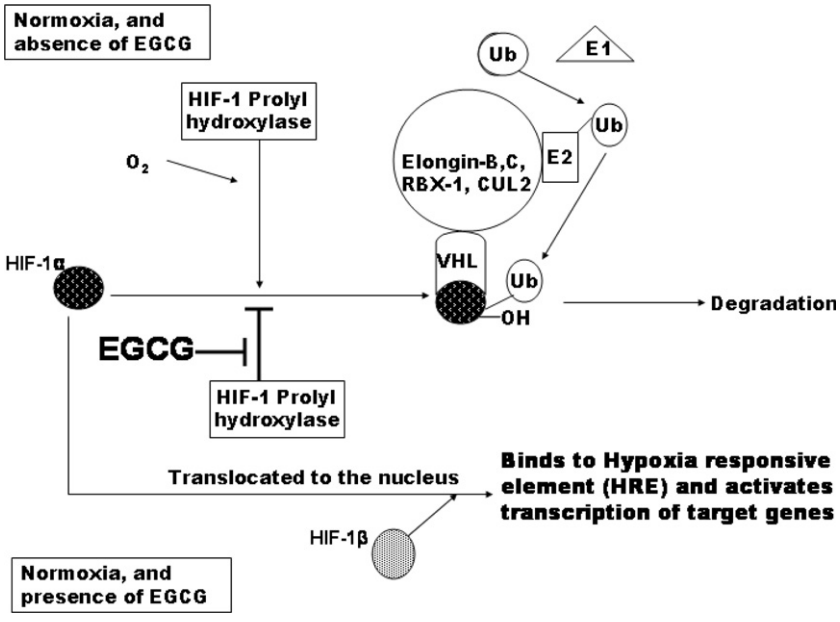

Fig. 6. Working model of EGCG's mechanism of action in increasing HIF-1 transcriptional activity. Under normoxic conditions and in the absence of EGCG, HIF-1α is hydroxylated at Pro^{402} and Pro^{564} within the HIF-1α ODD by Fe^{2+}-dependent HIF-1 prolyl hydroxylase thereby allowing for HIF-1α recognition by an E3 ubiquitin ligase complex. pVHL, the substrate recognizing component of the E3 ubiquitin ligase complex, links the hydroxylated HIF-1α to the ubiquitination machinery and proteasome-mediated degradation. Under normoxic conditions and in the presence of EGCG, HIF-1 prolyl hydroxylase is inhibited by EGCG, thereby resulting in the accumulation of HIF-1α which translocates to the nucleus and associates with HIF-1β subunit. The HIF-1αβ heterodimer binds to hypoxia response element found in the promoter/enhancer regions of HIF-1 target genes and activates their expression.

Hydroxylation of Asn^{803} prevents the interaction of HIF-1α with co-activators p300/CBP [20,21]. We are currently investigating whether EGCG inhibits FIH-1 activity and Asn^{803} hydroxylation.

In summary, we demonstrated for the first time that EGCG upregulated HIF-1α transcription factor via direct inhibition of prolyl hydroxylation and subsequent pVHL interaction, leading to increase in HRE promoter activity.

References

[1] G.L. Semenza, Targeting HIF-1 for cancer therapy, Nat. Rev. Cancer 3 (2003) 721–732.
[2] A.C. Epstein, J.M. Gleadle, L.A. McNeill, K.S. Hewitson, J. O'Rourke, D.R. Mole, M. Mukherji, E. Metzen, M.I. Wilson, A. Dhanda, Y.M. Tian, N. Masson, D.L. Hamilton, P. Jaakkola, R. Barstead, J. Hodgkin, P.H. Maxwell, C.W. Pugh, C.J. Schofield, P.J. Ratcliffe, *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation, Cell 107 (2001) 43–54.
[3] P. Jaakkola, D.R. Mole, Y.M. Tian, M.I. Wilson, J. Gielbert, S.J. Gaskell, A. Kriegsheim, H.F. Hebestreit, M. Mukherji, C.J. Schofield, P.H. Maxwell, C.W. Pugh, P.J. Ratcliffe, Targeting of HIF-alpha to the von Hippel–Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation, Science 292 (2001) 468–472.
[4] N. Masson, C. Willam, P.H. Maxwell, C.W. Pugh, P.J. Ratcliffe, Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation, EMBO J. 20 (2001) 5197–5206.
[5] M. Ivan, K. Kondo, H. Yang, W. Kim, J. Valiando, M. Ohh, A. Salic, J.M. Asara, W.S. Lane, W.G. Kaelin Jr., HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing, Science 292 (2001) 464–468.
[6] R.K. Bruick, S.L. McKnight, A conserved family of prolyl-4-hydroxylases that modify HIF, Science 294 (2001) 1337–1340.
[7] R.K. Bruick, Oxygen sensing in the hypoxic response pathway: regulation of the hypoxia-inducible transcription factor, Genes Dev. 17 (2003) 2614–2623.
[8] R.K. Bruick, S.L. McKnight, Transcription. Oxygen sensing gets a second wind, Science 295 (2002) 807–808.
[9] M.E. Cockman, N. Masson, D.R. Mole, P. Jaakkola, G.W. Chang, S.C. Clifford, E.R. Maher, C.W. Pugh, P.J. Ratcliffe, Maxwell, Hypoxia-inducible factor-alpha binding and ubiquitylation by the von Hippel–Lindau tumor suppressor protein, J. Biol. Chem. 275 (2000) 25733–25741.
[10] M. Ohh, C.W. Park, M. Ivan, M.A. Hoffman, T.Y. Kim, L.E. Huang, N. Pavletich, V. Chau, W.G. Kaelin, Ubiquitination of hypoxia-inducible factor requires direct binding to the β-domain of the von Hippel–Lindau protein, Nat. Cell Biol. 2 (2000) 423–427.
[11] K. Tanimoto, Y. Makino, T. Pereira, L. Poellinger, Mechanism of regulation of the hypoxia-inducible factor-1α by the von Hippel–Lindau tumor suppressor protein, EMBO J. 19 (2000) 4298–4309.
[12] B.H. Jiang, E. Rue, G.L. Wang, R. Roe, G.L. Semenza, Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor-1, J. Biol. Chem. 271 (1996) 17771–17778.
[13] Y. Kuroda, Y. Hara, Antimutagenic and anticarcinogenic activity of tea polyphenols, Mutat. Res. 436 (1999) 69–97.
[14] Y. Cao, R. Cao, Angiogenesis inhibited by drinking tea, Nature 398 (1999) 381.
[15] S. Nam, D.M. Smith, Q.P. Dou, Ester bond-containing tea polyphenols potently inhibit proteasome activity in vitro and in vivo, J. Biol. Chem. 276 (2001) 13322–13330.
[16] C.S. Yang, Z.Y. Wang, Tea and cancer, J. Natl. Cancer Inst. 85 (1993) 1038–1049.
[17] P.H. Maxwell, M.S. Wiesener, G.W. Chang, S.C. Clifford, E.C. Vaux, M.E. Cockman, C.C. Wykoff, C.W. Pugh, E.R. Maher, P.J. Ratcliffe, The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis, Nature 399 (1999) 271–275.
[18] M. Wang, M.E. Stearns, Isolation and characterization of PC-3 human prostatic tumor sublines which preferentially metastasize to select organs in S.C.I.D. mice, Differentiation 48 (1991) 115–125.
[19] Y.D. Zhou, Y.P. Kim, X.C. Li, S.R. Baerson, A.K. Agarwal, T.W. Hodges, D. Ferreira, D.G. Nagle, Hypoxia-inducible factor-1 activation by (−)-epicatechin gallate: potential adverse effects of cancer chemoprevention with high-dose green tea extracts, J. Nat. Prod. 67 (2004) 2063–2069.
[20] D. Lando, D.J. Peet, D.A. Whelan, J.J. Gorman, M.L. Whitelaw, Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch, Science 295 (2002) 858–861.
[21] D. Lando, D.J. Peet, J.J. Gorman, D.A. Whelan, M.L. Whitelaw, R.K. Bruick, FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor, Genes Dev. 16 (2002) 1466–1471.