Inhibition of the Catalytic Activity of Hypoxia-Inducible Factor-1α-Prolyl-Hydroxylase 2 by a MYND-Type Zinc Finger

Kyung-Ok Choi, Taekyong Lee, Naery Lee, Ji-Hyun Kim, Eun Gyeong Yang, Jung Min Yoon, Jin Hwan Kim, Tae Gyu Lee, and Hyunsung Park

Department of Life Science, University of Seoul, Seoul, Korea (K.-O.C., T.L., N.L., H.P.); Life Science Division, Korea Institute of Science and Technology, Seoul, Korea (J.-H.K., E.G.Y.); and the Division of Drug Discovery, CrystalGenomics, Inc., Daejeon City, Korea (J.M.Y., J.H.K., T.G.L.)

Received May 26, 2005; accepted September 9, 2005

ABSTRACT

Hypoxia-induced gene expression is initiated when the hypoxia-inducible factor-1 (HIF-1) α subunit is stabilized in response to a lack of oxygen. An HIF-1α-specific prolyl-hydroxylase (PHD) catalyzes hydroxylation of the proline-564 and/or -402 residues of HIF-1α by an oxygen molecule. The hydroxyproline then interacts with the ubiquitin E3 ligase von Hippel Lindau protein and is degraded by an ubiquitin-dependent proteasome. PHD2 is the most active of three PHD isoforms in hydroxylating HIF-1α. Structural analysis showed that the N-terminal region of PHD2 contains a Myeloid translocation protein 8, Nervy, and DEAF1 (MYND)-type zinc finger domain, whereas the catalytic domain is located in its C-terminal region. We found that deletion of the MYND domain increased the activity of both recombinant PHD2 protein and in vitro-translated PHD2. The zinc chelator N,N,N’,N’-tetakis(2-pyridylmethyl)ethylenediamine augmented the activity of wild-type PHD2-F but not that of PHD2 lacking the MYND domain, confirming that the zinc finger domain is inhibitory. Overexpression of PHD2 lacking the MYND domain caused a greater reduction in the stability and function of HIF-1α than did overexpression of wild-type PHD2, indicating that the MYND domain also inhibits the catalytic activity of PHD2 in vivo.

Hypoxia is the most common type of cell injury in various human diseases, including myocardial infarction, stroke, acute renal failure, and solid tumors. However, organisms have evolved mechanisms for adapting to hypoxia. Thus, hypoxia leads to up-regulation of the transcription of genes involved in anaerobic ATP production and oxygen delivery. Hypoxia-inducible factor-1 (HIF-1) is a widespread transcription factor that promotes expression of hypoxia-inducible genes such as vascular endothelial growth factor, erythropoietin, glucose transporters, and glycolytic enzymes (Seagroves et al., 2001; Masson and Ratcliffe, 2003). It consists of HIF-1α and HIF-1β subunits, both of which belong to the basic helix-loop-helix-Per-Arnt-Sim family. Arnt (HIF-1β) is a partner of the aryl hydrocarbon receptor as well as of HIF-1α and other basic helix-loop-helix-Per-Arnt-Sim proteins. HIF-1α is rapidly degraded under normoxic condition by the ubiquitin-proteasome system, whereas the level of Arnt is constant (Huang et al., 1998; Kallio et al., 1999). Hydroxylation of proline-564 and/or -402 residues in the oxygen-dependent degradation domain (ODD) of HIF-1α initiates its ubiquitination and subsequent proteasomal degradation (Masson and Ratcliffe, 2003). Prolyl-hydroxylation of HIF-1α is catalyzed by a novel HIF-1α-specific prolyl-hydroxylase that requires O2, 2-oxoglutarate, vitamin C, and Fe2+ (Bruck and McKnight, 2001; Epstein et al., 2001; Ivan et al., 2001; Jaakkola et al., 2001). The tumor suppressor von Hippel-Lindau (VHL) protein, which functions as an E3 ubiquitin ligase, interacts with the hydroxylated prolines of HIF-1α and brings about the assembly of a complex that activates a ubiquitin-dependent proteasome (Maxwell et al., 1999; Ohh et al., 2000; Min et al., 2002). When cells lack oxygen, proline hydroxylation ceases, and HIF-1α protein accumulates. In mammalian cells, a family of HIF-1α-specific prolyl-4-hydroxylases have been identified and given the abbreviations PHD1 (PHP3, EGLN2), PHD2 (PHP2, EGLN1), and PHD3.
Akt have been reported to activate HIF-1 and inhibit the catalytic activity of PHDs, as well as other agents (Masson and Ratcliffe, 2003; Masson et al., 2004).

In addition to hypoxia, Co(II) ion, and iron chelators, which inhibit the catalytic activity of PHDs, as well as other agents such as growth factors and the oncogenes Ras, active Src, and Akt have been reported to activate HIF-1α under normoxia (Zundel et al., 2000; Chan et al., 2002; Karni et al., 2002). It is not clear whether these nonhypoxic stimuli repress the catalytic activity of PHD2, to stabilize HIF-1α, or act in some other way. In this study, we investigated whether the activity of PHD2 is regulated. By analyzing the catalytic activity of purified PHD2 and truncated mutants, we found that the N-terminal region of PHD2 contains a MYND-type zinc finger domain that inhibits catalytic activity.

**Materials and Methods**

**Cells, cDNAs, and Reagents.** Human epithelial HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (Cambrex Bio Science Walkersville, Inc., Walkersville, MD), gentamicin (5 μg/ml), Invitrogen, Carlsbad, CA), and Fungizone (0.25 μg/ml; Invitrogen) in humidified air containing 5% CO2 at 37°C. Cells were made hypoxic by incubation in an anaerobic incubator (model 1029; Thermo Electron Corporation, Waltham, MA) in 5% CO2, 10% H2, and 85% N2 at 37°C or in a Multi-gas incubator (model NU-4950G; NuAire, Inc., Plymouth, MN). We used the following human cDNAs in expression vectors, transfection assays, and in vitro transcription and translation experiments: PHD1 (AJ310544), PHD2 (AJ310543), PHD3 (AJ310545), HIF-1α (U22431), and VHL (AF010238). The pHRD/HLuc reporter plasmid contained four copies of the erythropoietin hypoxia-responsive element 5'-GATCGGCTTACCTGTTGCTGTCCTA-3'; nucleotides 3449 to 3470. Anti-HIF-1α was obtained from BD Transduction Laboratories (Lexington, KY). We obtained N,N,N',N'-tetrais(2-pyridylmethyl)ethylenediamine (TPEN) from Calbiochem (San Diego, CA), and all remaining chemicals were from Sigma Chemical (St. Louis, MO). Culture media were purchased from Invitrogen, and fetal bovine serum was from Cambrex Bio Science Walkersville, Inc.

**Expression of PHD2 and HIF-1α.** Full-length cDNAs for PHD1, -2, and -3 were cloned from a human lymphocyte cDNA library into pcDNA3.1B (+) (Invitrogen). For in vitro transcription and translation, wild-type PHD2 (PHD2-F) cDNA and cDNAs for PHD2-182 (amino acids 182–426), PHD2-60 (amino acids 60–426), PHD2-16 (amino acids 16–426), and PHD2-184 (amino acids 184–418) were subcloned into pcDNA3.1B (+) (Invitrogen) or pET21bHis2 (Novagen, Madison, WI). For bacterial expression, the cDNAs for PHD2-F and the catalytic domain PHD2-184 (amino acids 184–418) were subcloned into pET21bHis2 (+) vector (Novagen) and expressed with C-terminal histidine tags. For transfections, cDNAs for PHD2-F and PHD2-60 were subcloned into pCMV-3xFLAG vector (Sigma Chemical) and expressed with N-terminal FLAG tags. We subcloned VHL into pcDNA 3.1/hygro for in vitro transcription and translation. A plasmid encoding the HIF-α 401- to 603-amino acid region [the ODD linked to glutathione S-transferase (GST)] was kindly provided by Dr. Seong-Eon Ryu (Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea). Peptides [Biotin-DLDDLELAPYP-MDDDFQLR and Biotin-DLDDLELAP/0H/YIPMDDDDFQLR] were synthesized by AnyGen Co. Ltd. (Kwangju, Korea). These 20-mer peptides contain residues 556 to 575 of HIF-1α.

**Expression and Purification of PHD2 Protein.** The human PHD2 gene (identical to AJ310543) was cloned into the pET21b His2 (+) vector and overexpressed in Escherichia coli as histidine-tagged fused proteins and purified by Ni2+–affinity chromatography. The histidine fusions of full-length PHD2-F (amino acids 1–426) and catalytic domain PHD2-184 (amino acids 184–418) were further purified by gel-filtration chromatography (HiLoad Superdex200; GE Healthcare, Little Chalfont, Buckinghamshire, UK) and concentrated by ultrafiltration. PHD1, -2, and -3 or mutants of PHD2 pcDNA3.1B (+) were in vitro transcribed and translated from the T7 promoter using a rabbit reticulocyte lysate (Promega, Madison, WI).

**Measurement of PHD Activity by a VHL Pull-Down Assay.** The in vitro VHL pull-down assay was performed as described by Jaakkola et al. (2001). In brief, [35S]methionine-labeled VHL protein was synthesized by in vitro transcription and translation using the pcDNA3.1/hygro-VHL plasmid, according to the instruction manual (catalog no. L1170, Promega). GST-ODD (amino acids 401–603 of human HIF-1α) was expressed in E. coli and purified with glutathione–uniflow resin according to the instruction manual (catalog no. 8912-1; BD Biosciences Clontech, Palo Alto, CA). Resin-bound GST-
ODD (200 μg of protein/80 μl of resin volume) was incubated in the presence of 2 mM ascorbic acid, 100 μM FeCl₃, and 5 mM α-ketoglutarate with the indicated amounts of enzyme in 200 μl of NETN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride) with mild agitation for 90 min at 30°C. The reaction mixture was centrifuged and washed three times with 10 volumes of NETN buffer. Resin-bound GST-ODD was mixed with 10 μl of 35S-labeled VHL in 500 μl of EBC buffer (120 mM NaCl, 50 mM Tris-HCl, pH 8.0, and 0.5% (v/v) Nonidet P-40). After mild agitation at 4°C for 2 h, the resin was washed three times with 1 ml of NETN buffer, and proteins were eluted at 3X SDS sample buffer, fractionated by 12% SDS-PAGE, and detected by autoradiography. The amount of each sample loaded was monitored by staining the GST-ODD with Coomassie Blue.

Mass Spectrophotometric Analysis. HIF-1α peptide (Biotin-DLDDLMEAPYIHMDDDFQLR) (400 ng) was incubated with 2 μg of PHD2-184 in a final volume of 10 μl in NETN buffer containing 5 mM ascorbic acid, 100 μM FeCl₃, and 5 mM α-ketoglutarate at 30°C for 90 min. For matrix-assisted laser desorption ionization/time of flight (MALDI-TOF) analyses, α-cyano-4-hydroxycinnamic acid solution was prepared in acetonitrile/water containing 0.1% trifluoroacetic acid [50:50 (v/v)] at a concentration of 10 mg/ml. This matrix solution was used to dilute samples (1:10 ratio) to a final concentration of 1 ng/μl. They were then spotted directly onto the target plate and allowed to air dry. Mass spectrometric analyses of the samples were performed with a Voyager analyzer (Applied Biosystems, Foster City, CA).

Results

The Catalytic and Inhibitory Domains of PHD2. We examined the hydroxylation activity of the PHDs by measur-

---

### A

**Consensus pattern**

\[ \text{CH} \cdot X(2.4) \cdot C \cdot X(7.17) \cdot C \cdot X(0.2) \cdot C \cdot X(4) \cdot [YFT] \cdot C \cdot X(3) \cdot [CH] \cdot X(6.9) \cdot H- \cdot X(3.4) \cdot C \]

---

**Fig. 2.** Comparison of the activities of PHD2 deletion mutants. A, structural analysis using Exipay programs (http://us.expasy.org). The N-terminal region of PHD2 (amino acids 21–58) contains a MYND-type zinc finger domain. The C-terminal region has the conserved catalytic domain (amino acids 294–392) of 2-oxoglutarate and Fe(II)-dependent dioxygenases such as collagen prolyl-4-hydroxylase (amino acids 205–392). The consensus sequences among MYND-type zinc finger proteins PHD2, MTSR, MTG8, DEAF, BS69, and PDC2 are shown. The deletion mutants of PHD2 are shown. B, hydroxylation and VHL pull-down activity of the PHD2 mutants. Each PHD2 deletion mutant was synthesized by in vitro transcription and translation using rabbit reticulocyte lysate. Resin-bound GST-ODD was incubated with 15 μl of each PHD-programmed rabbit reticulocyte lysate. Prolyl-hydroxylation of HIF-1α was measured by VHL pull-down as described under Materials and Methods. The amount of each sample loaded was monitored by measuring GST-ODD stained with Coomassie Blue. C, HIF-1α 20-mer peptide (Biotin-DLDDLMEAPYIHMDDDFQLR) was incubated with 15 μl of each PHD-programmed rabbit reticulocyte lysate. Prolyl-hydroxylation of HIF-1α was measured by VHL pull-down as described under Materials and Methods. One representative of three independent experiments with similar results is shown. D, expression levels of the in vitro-translated PHD mutants. 35S-Labeled PHD2 mutants were synthesized by in vitro transcription and translation. To estimate the amounts of the synthesized PHDs, 8 μl of each programmed lysate was analyzed by 12% SDS-PAGE followed by autoradiography. PHD2-184 (amino acids 184–418) was expressed as a histidine-tagged protein with an additional 40 amino acids between residue 418 of PHD2 and the histidine tag. One representative of three independent experiments with similar results is shown.
incubation with PHD2-184 protein (400 ng) as described under Materials and Methods. D, test for reversibility of PHD2 activity. One hundred nanograms of PHD2-184 recombinant protein and S-100 fraction of HeLa cell extract (1 mg of microsomal proteins) that has the HIF-1\(\alpha\) activity was added to the treated bead-bound GST-ODD, and the captured \(^{35}\)S-labeled VHL was visualized by SDS-PAGE and autoradiography. Hydroxylation was performed in normoxic conditions for 30 min, and the reaction mixtures were then incubated in anaerobic conditions (0.1% \(O_2\), 5% \(CO_2\), 85% \(N_2\), and 10% \(H_2\)) for the indicated times. All reactions were performed in the presence of 100 \(\mu\)M ferrous chloride, 2 mM ascorbic acid, and 5 mM \(\alpha\)-ketoglutarate at 30°C. Ten microliters of labeled VHL was added with the treated bead-bound GST-ODD, and the captured \(^{35}\)S-labeled VHL was visualized by SDS-PAGE and autoradiography. Hydroxylation was performed in normoxic conditions for 30 min, and the reaction mixtures were then incubated in anaerobic conditions (0.1% \(O_2\), 5% \(CO_2\), 85% \(N_2\), and 10% \(H_2\)) for the indicated times. All reactions were performed in the presence of 100 \(\mu\)M ferrous chloride, 2 mM ascorbic acid, and 5 mM \(\alpha\)-ketoglutarate at 30°C. Ten microliters of labeled VHL was added with the treated bead-bound GST-ODD, and the captured \(^{35}\)S-labeled VHL was visualized by SDS-PAGE and autoradiography.
recombinant PHD2-184 by detecting hydroxylation of HIF-1α rather than by visualizing captured VHL, we incubated bio-
tinylated HIF-1α peptide (amino acids 556–575) with PHD2-
184 and determined the change in molecular weight of the
peptide MALDI-TOF analysis. Because the peptide contains
proline-564, hydroxylation by PHD2-184 increases its molecu-
lar weight. HIF-1α peptide samples treated with PHD2-184
showed a second MALDI-TOF peak that corresponded to an
increase in molecular weight of 16 (Fig. 3C). This confirms
that the recombinant PHD2-184 hydroxylates HIF-1α with
out any other cellular components.

We tested whether the hydroxylation reaction can be re-
versed. Immobilized HIF-1α was treated with recombinant
PHD2-184 or S-100 fraction of HeLa cells in normoxia for 30
min and then further incubated in anoxic condition for the
indicated times. VHL pull-down analysis (Fig. 3D) indicated
that lack of oxygen did not reverse the hydroxylation reaction
(Masson et al., 2001; Chan et al., 2002). This finding suggests
that, to stabilize HIF-1α, hypoxia can reduce the interaction
of VHL with newly synthesized HIF-1α but cannot reverse
the hydroxylation of pre-existing HIF-1α.

Effects of Zinc Chelator TPEN on the Activity of
PHD2. To confirm the inhibitory action of the MYND-type
zinc finger domain, we treated recombinant PHD2-F and
PHD2-184 proteins with the zinc-specific chelator TPEN and
measured their activities. Hydroxylation and VHL pull-down
analysis indicated that TPEN increased the activity of recom-
binant PHD2-F but not that of PHD2-184 (Fig. 4, A and C). It
also increased the activity of in vitro-transcribed and -trans-
lated PHP2-F but not of PHD2-184 or PHD2-60, which lack the
MYND-type zinc finger domain (Fig. 4B). Although TPEN
can also act as an iron-chelating agent, this did not
affect the activity of the PHD2 mutants because an excess of
iron (100 μM) was present in the reaction mixtures together
with the TPEN (2 or 5 μM). Moreover, the addition of zinc
ions reversed the effect of TPEN on PHD2-F (Fig. 4, A–C).
These observations imply that chelating Zn(II) with TPEN
activates the enzyme by incapacitating the MYND-type zinc
finger domain.

Effect of the MYND Zinc Finger Domain on the Sta-
tability and Transactivation of HIF-1α. The finding that
the MYND domain inhibits hydroxylation of HIF-1α and its
interaction with VHL suggested that deletion of the MYND
domain would increase VHLD-dependent ubiquitination and
degradation of HIF-1α. HeLa cells were transfected with
enough HIF-1α plasmid (1 μg) to overcome hydroxylation/
VHL/ubiquitin-dependent degradation, and Western analy-
ysis showed that HIF-1α protein could be detected even in
normoxic condition. Cotransfection with a limited amount
(500 ng) of FLAG-tagged PHD2-F plasmid reduced the level
of HIF-1α slightly, whereas cotransfection with the same
amount of PHD2-60 had a greater effect in both normoxia
(21% O2) and partial hypoxia (5% hypoxia), indicating that
deletion of the MYND domain increases hydroxylation/VHL-

Fig. 4. Effects of TPEN on PHD2 activities. A, 50 ng of
the recombinant PHD2-F or PHD2-184 was preincu-
bated with 2 or 5 μM TPEN or 5 μM TPEN plus 10 μM
ZnCl2 for 10 min at room temperature. The treated
PHD2-F or PHD2-184 was then added to Biotin-HIF-1α
(556-575) peptide and incubated as described under Ma-
terials and Methods. The captured 35S-labeled VHL was
visualized by SDS-PAGE and autoradiography. One repre-
sentative of at least two independent experiments with
similar results is shown. B, indicated amount of in vitro-
translated PHD2-F, PHD2-60, or PHD2-184 was preincu-
bated with 2 or 5 μM TPEN or 5 μM TPEN plus 10 μM
ZnCl2 for 10 min at room temperature. They were then
added to biotin-HIF-1α (556-575) peptide and incubated
as described under Materials and Methods. The captured
35S-labeled VHL was visualized by SDS-PAGE and au-

toradiography. C, recombinant PHD2-F protein (1 μg)
was preincubated with 2 or 5 μM TPEN or 5 μM TPEN
plus 10 μM ZnCl2 for 10 min at room temperature. They
were then added to resin-bound GST-ODD and incu-
bated in the presence of 100 μM ferrous chloride, 2 mM
vitamin C, and 5 mM α-ketoglutarate for 90 min at 30°C.
After washing with NETN buffer, 10 μl of 35S-labeled
VHL was added, and incubation continued at 4°C for 2 h.
Captured VHL was visualized by SDS-PAGE and au-

toradiography. The amount of each sample loaded was
monitored by measuring GST-ODD stained with Coom-
assie Blue. One representative of two independent ex-
periments with similar results is shown.

\[\text{HIF-1}\alpha \text{(556-575)} \]
decreased the expression of an HIF-1α-driven reporter gene (Fig. 6), demonstrating that the presence of the MYND domain limits the hydroxylation/VHL-dependent degradation of HIF-1α in vivo.

**Discussion**

We have shown that purified recombinant PHD2 can hydroxylate HIF-1α without needing any other polypeptides, unlike collagen proline hydroxylase, which consists of two α chains and two β chains. In agreement with several other studies, we confirmed that PHD2 is the major HIF-1α-prolyl-4-hydroxylase. PHD2 shares the conserved catalytic domain of 2-oxoglutarate and Fe(II)-dependent dioxygenases with other prolyl-4-hydroxylases, including PHD1, PHD3, and collagen prolyl hydroxylase, but it has a unique N-terminal MYND-type zinc finger domain. We have demonstrated that deletion of the MYND-type zinc finger domain increases the activity of both in vitro-translated PHD2 and recombinant PHD2 protein (Figs. 2 and 3) and that treatment with the zinc chelator TPEN increases the activity of PHD2-F but not PHD2 mutants, which lack the MYND domain (Fig. 4), indicating that the catalytic activity of PHD2 is inhibited by the N-terminal zinc finger domain. Our transfection analyses demonstrated that deletion of the MYND domain destabilized HIF-1α under both normoxia and hypoxia (5% O₂) and decreased the expression of an HIF-1α-driven reporter gene. These results suggest that the MYND domain inhibits the hydroxylation activity of PHD2 and the resulting VHL-dependent degradation of HIF-1α in vivo (Fig. 7). It will be of
interest to determine whether the mutations affecting the MYND domain found in certain human diseases affect the activity of PHD2 and the stability of HIF-1.

The reduction in the stability of HIF-1α caused by deletion of MYND domain in vivo was small compared with the reduction in enzyme activity in vitro. This reflects the fact that hydroxylation of HIF-1α may not be a limiting step for its ubiquitin-dependent degradation in vivo. Because the MYND domain inhibits the catalytic activity of pure recombinant PHD2, this domain may reduce the accessibility of its catalytic domain. Although more work needs to be done, the results of a yeast two-hybrid screen suggest that the MYND domain of PHD2 does not interact with the catalytic domain but rather with a component of a specialized cytoplasmic organelle (J. Lee, unpublished data).

MYND is an acronym for the three best-characterized representatives: Myeloid translocation protein 8 (MTG8/ETO) (Wang et al., 1998), Nervy protein, and Def-1. The MYND-type zinc finger contains eight amino acids that can coordinate two zinc atoms (Fig. 2A). The common function of this domain is not clear, but many of the proteins, including MTG8/ETO (Lutterbach et al., 1998; Wang et al., 1998), BS69 (Ansieau and Leutz, 2002), m-Bop (Gottlieb et al., 2002), and Mammalian programmed cell death protein 2 (PDCD2/RP8PDCD2) (Scarr and Sharp, 2002), are known to be transcriptional repressors. MTG8 is part of a high-molecular-weight complex that contains corepressors and histone deacetylases (Lutterbach et al., 1998; Wang et al., 1998), whereas BS69 is an adenovirus E1A binding protein that binds to the transcription activation domain of the adenovirus type 5 E1A 32-kDa protein (289R) and inhibits its transcriptional activation (Ladendorff et al., 2001). The MYND domain of BS69 interacts with the PXLXP motifs of several other cellular and oncoviral proteins, including Epstein-Barr virus EBNA2 and Myc-related cellular protein MGA as well as c-Myc (Ansieau and Leutz, 2002). Its MYND domain also interacts with a corepressor, N-CoR, and is a component of several transcriptional repressor complexes (Masselink and Bernards, 2000). Bop is expressed specifically in cardiact and muscle precursor cells and mediates chromatin modification as a histone deacetylase-dependent repressor essential for cardiogenesis (Gottlieb et al., 2002). The MYND domain of Bop also interacts with muscle-specific transcription factor nascent polypeptide-associated complex skNAC. The PXLXP motif of skNAC is required for interaction with MYND domain of m-Bop (Wang et al., 1998). The finding that the MYND domains of several proteins are involved in interactions with PXLXP motifs suggests that the same may be true of the MYND-type domain of PHD2.

The N-terminal MYND domain of PHD2 has an inhibitory effect on the C-terminal catalytic activity, and many MYND domains are involved in protein-protein interactions, suggesting that the catalytic activity of PHD2 may be modulated by a cellular factor that interacts with the MYND domain of PHD2.

Acknowledgments

We thank Dr. Kyu-Won Kim (Seoul National University) and Seoung-Eon Ryu (Korea Research Institute of Bioscience and Biotechnology) for providing pCMV/myc(3B)-VHL and GST-ODD.

References


