

The Regulation of Hypoxia Inducible Factor (HIF)1 α Expression by Quercetin: an In Silico Study

Sri Puji Astuti Wahyuningsih¹,
Firli Rahmah Primula Dewi¹,
Amy Saik Yi Hsan², Looi Mee
Lee³, Vuanghao Lim⁴, Lionel
In Lian Aun⁵, Tau Chuan
Ling⁶, Sephia Tiara Marviella¹

¹Department of Biology, Faculty of Science
and Technology, Universitas Airlangga,
Surabaya, Indonesia

²Department of Pre-Clinical Sciences,
Faculty of Medicine and Health Sciences,
University Tunku Abdul Rahman, Malaysia

³Department of Biomedical Science,
Faculty of Medicine, University of Malaya,
Malaysia

⁴Advanced Medical and Dental Institute,
Universiti Sains Malaysia, Malaysia

⁵Department of Biotechnology, Faculty
of Applied Sciences, UCSI Kuala Lumpur,
Malaysia.

⁶Institute of Biological Sciences, Faculty of
Science, University of Malaya, Malaysia

Corresponding author: Firli Rahmah Primula
Dewi, PhD, Faculty of Science and Technology,
Universitas Airlangga. Address: Jl. Ir. Dr.
Soekarno, Mulyorejo Surabaya, Indonesia.
Phone: +6281356596292. E-mail address: firli.
rahmah@fst.unair.ac.id. ORCID ID: [https://orcid.
org/0000-0002-7813-8435](https://orcid.org/0000-0002-7813-8435).

doi: 10.5455/aim.2022.30.96-99

ACTA INFORM MED. 2022 JUN 30(2): 96-99

Received: May 03, 2022

Accepted: Jun 02, 2022

© 2022 Sri Puji Astuti Wahyuningsih, Amy Saik Yi
Hsan, Looi Mee Lee, Vuanghao Lim, Lionel In Lian
Aun, Sephia Tiara Marviella, Firli Rahmah Primula
Dewi

This is an Open Access article distributed under the
terms of the Creative Commons Attribution Non-
Commercial License ([http://creativecommons.org/
licenses/by-nc/](http://creativecommons.org/licenses/by-nc/)) which permits unrestricted non-
commercial use, distribution, and reproduction in any
medium, provided the original work is properly cited.

ABSTRACT

Background: Cancer disease is a growing health problem in developing and developed countries. Hypoxia-inducible factor-1 α (HIF1 α) is a transcription factor responsible for expressing several proteins involved in angiogenesis. Quercetin can suppress HIF1 α expression due to the inhibition of protein synthesis. However, to date, the study exploring the potential of quercetin in repressing HIF1 α through its degradation mechanism has never been done. An in silico study is needed as a preliminary study to understand the mechanism underlining this possibility. **Objective:** This study aimed to investigate the potential of quercetin in regulating HIF1 α expression through the ubiquitin degradation pathway by in silico study. **Methods:** This study was performed by in silico analysis, including biological activity prediction, 3D protein structure collection, protein-ligand and protein-protein docking, and the visualization of the docking results. **Results:** The probability activity (Pa) score of quercetin as an HIF1 α expression inhibitor was 0.969. In the absence of quercetin, the center-weighted score of HIF1 α - pVHL, HIF1 α - FIH, and HIF1 α - PHD2 was -699.4 kJ/mol, -846.0 kJ/mol, and -650.5 kJ/mol, respectively. In the presence of quercetin, the weighted score of HIF1 α - pVHL, HIF1 α - FIH, and HIF1 α - PHD2 was reduced to -728.1 kJ/mol, -854.2 kJ/mol, and -650.5 kJ/mol, respectively. **Conclusion:** Quercetin could directly promote HIF1 α and pVHL interaction, thus increasing the degradation of HIF1 α by ubiquitin-dependent pathway.

Keywords: Cancer disease, HIF1 α , in silico, pVHL, quercetin.

1. BACKGROUND

Cancer is a growing health problem in both developing and developed countries. According to the World Health Organization (February 2014), 8.2 million patients died from cancer in 2012. It has also been estimated that the number of annual cancer cases will have increased from 14 million in 2012 to 22 million within the next two decades. Cancer arises due to the alteration of the DNA sequence, which enables the cells to proliferate abnormally (1). Many of these mutations give a cell the ability to sustain proliferation and immortality, suppress apoptosis, and induce angiogenesis and metastasis (2).

The use of natural compounds for cancer prevention and therapy has been an interesting area of study in the last decades (3-5). Quercetin is a

flavonoid present in many plants (6). Due to its antioxidant, anti-tumor, and anti-inflammatory activity, quercetin has been studied extensively as a chemoprevention agent in several cancer models. Quercetin has been shown to inhibit the proliferation of a wide range of cancer types such as prostate, cervical, lung, breast, and colon (7). HIF1 α protein is subject to degradation during normoxia by the ubiquitin-proteasome pathway mediated by pVHL.

The association of HIF1 α and pVHL is regulated by post-translational modification of proline residue that is mediated by prolyl hydroxylase (PHD) and asparagine residue mediated by factor inhibiting HIF1 α (FIH). FIH hydroxylase one asparagine residue in the C-terminal transactivation domain (C-TAD) of HIF1 α . In addition, hydroxylation of

HIF1 α by FIH blocks the association of HIF-1 with its transcriptional co-activators CBP/p300, thus inhibiting the transcriptional of the downstream target of HIF1 α (8, 9).

A previous study reported quercetin suppressed the hypoxia-inducible factor-1 α (HIF1 α) accumulation during hypoxia in human prostate cancer LNCaP, colon cancer CX-1, and breast cancer SkBr3 cells. A previous study reported that suppression of HIF1 α accumulation during treatment with quercetin under hypoxic conditions is due to inhibition of protein synthesis (10). However, to date, the study exploring the potential of quercetin in repressing HIF1 α through its degradation mechanism has never been done. Hence an in silico study is needed as a preliminary study to examine the possibility of quercetin in suppressing HIF1 α through its degradation mechanism pathway.

2. OBJECTIVE

The aim of this study was to investigate the potential of quercetin in regulating HIF1 α expression through ubiquitin degradation pathway by in silico study.

3. MATERIAL AND METHODS

Biological Activity Prediction

The biological activity of quercetin was predicted using the Prediction of Activity Spectra for Substances (PASS) Server (<http://www.pharmaexpert.ru/passonline/>) (11). The compounds were predicted for human intestinal absorption (HIA) for evaluating the potency for oral use by using the Laboratory of Molecular Modeling and Design webserver (<http://lmmdd.ecust.edu.cn>).

The lethal dose (LD50) of quercetin was also evaluated to predict the lethal dose when applied in vivo in a rat model animal (12).

Ligand-receptor	Cluster	Cluster-Rank	Energy (kJ/mol)	Fullfitness	DG
Quercetin - pVHL	22	0	38.996	-2147.146	-7.803
Quercetin - FIH	1	3	37.104	-1847.347	-8.334
Quercetin - PHD2	0	3	30.885	-1236.019	-7.653

Table 1. Interaction between quercetin and the protein regulator of HIF1 α

Obtaining 3D structure of HIF1 α , pVHL, FIH, and PHD2

The 3D structure of HIF1 α (1H2M), pVHL (1LM8), FIH (1IZ3), and PHD2 (2G1M) were obtained from RCSB Protein Data Bank (<https://www.rcsb.org/>). The protein were then prepared for docking by using UCSF Chimera (<https://www.cgl.ucsf.edu/chimera>) software.

Protein-ligand and protein-protein docking,

Docking of quercetin with pVHL, FIH, and PHD2 was performed by using the SwissDock webserver (<http://www.swissdock.ch>). The protein-protein docking simulation was then performed using ClusPro Webserver (<https://cluspro.org>) (13).

Visualization and Analysis of the Interactions

The docking results were visualized and analyzed by using UCSF Chimera and LigPlot+ (<https://www.ebi.ac.uk/thornton-srv/software/LigPlus>) software. The UCSF Chimera software was used to visualize the interaction site, while LigPlot+ was used to analyze hydrogen and hydrophobic binding between two molecules.

4. RESULTS

The biological activity of quercetin

To predict the molecular mechanism involving quercetin for cancer therapy, we first identify the probability activity of quercetin. Based on the probability activity

Ligand-Receptor	Cluster	Members	Weighted score (kcal/mol)	Interaction (bond)	Residue (Ligand - Receptor)
HIF1 α -pVHL	0	116	Center: -699.4 Lowest: -817.3	Hydrogen (2)	Ser343-Gln96; Arg306-Tyr112.
HIF1 α -pVHL, quercetin	0	128	Center: -728.1 Lowest: -875.0	Hydrogen (6)	Glu277-Arg113; Tyr276-Arg113; Asp283-Arg205; Leu282-Arg205; His286-Arg205; Gly312-Asn131.
HIF1 α -FIH	0	220	Center: -846.0 Lowest: -991.1	Hydrogen (12)	Arg440-Thr327; Arg440-Tyr325; Thr445-Gln320; Tyr450-Gln304; Gly420-Gln299; Asn448-Thr241; Asn463-Lys328; Arg366-Asp238; Arg362-Glu245; Arg379-Glu254; His378-Glu257; His378-Ser252.
HIF1 α -FIH, quercetin	0	206	Center: -854.2 Lowest: -997.3	Hydrogen (5)	Asp285-Lys315; Arg306-Tyr348; Asp283-Tyr348; Arg311-Glu328; Lys251-Pro303.
HIF1 α -PHD2	0	123	Center: -649.6 Lowest: -914.0	Hydrogen (15)	Tyr450-Glu318; Tyr450-Gln304; Asn448-Thr241; Arg362-Glu245; Arg362-Glu245; Gly420-Gln320; Thr445-Gln299; Arg379-Ser253; Arg440-Thr327; Arg440-Thr327; Arg440-Tyr325; Arg440-Tyr325; Asn463-Lys328; Arg379-Glu245; His378-Glu257.
HIF1 α -PHD2, quercetin	0	196	Center: -650.5 Lowest: -1004.8	Hydrogen (10)	Arg311-Arg295; Tyr314-Trp258; Tyr314-Arg322; Tyr314-Arg322; Tyr314-Asp315; Arg306-Gln239; Arg306-Gln239; Lys310-Arg396; Gln304-Arg252; Gln304-Arg252.

Table 2. The docking result of HIF1 α with its protein regulator in the absence or presence of quercetin.

(Pa) score, the three highest Pa score of quercetin was membrane integrity agonist (0.973), HIF1 α expression inhibitor (0.969), and peroxidase inhibitor (0.962). The screening was based on the Pa score. If the score of Pa is more than 0.7, the laboratory experiments result will be similar to computational prediction results. We performed HIA analysis to evaluate the pharmacokinetic properties of quercetin. The probability HIA+ score of quercetin was 0.9650, which means quercetin can be easily absorbed in the human intestine. The lethal dose parameter is essential information before conducting in vivo experiment. Lethal dose prediction analysis showed that quercetin has LD50 3.02 mol/kg.

Regulation of HIF1 α expression by quercetin

HIF1 α is the subunit of transcription factor HIF-1. Under the normoxic condition, HIF1 α is localized at cytoplasmic and will be targeted by ubiquitinylation degradation. However, under low concentrations of oxygen, the degradation of HIF-1 α is inhibited. The HIF1 α expression was regulated by hydroxylation of its two prolyls in the oxygen-dependent degradation domain (ODDD) by prolyl hydroxylase domain-containing protein 2 (PHD2) and one asparaginyl residue in the C-terminal transactivation domain (C-TAD) by factor-inhibiting HIF (FIH). This oxygen-dependent hydroxylation regulates the interaction with the von Hippel-Lindau tumor suppressor protein (pVHL). pVHL is the recognition component of an E3 ubiquitin ligase complex that targets HIF-1 α for proteolysis by the ubiquitin-proteasome pathway (14, 15).

In this study, we performed several docking analyses between HIF1 α with pVHL, FIH, and PHD2 in the presence or absence of quercetin. We first docked pVHL, FIH, and PHD2 with quercetin. The result shows that the energy for interaction between quercetin - pVHL was 38.996 kJ/mol, quercetin - FIH was 37.104 kJ/mol, and quercetin - PHD2 has the lowest binding energy with 30.885 kJ/mol (Table 1). Quercetin binds to FIH in the VHL interaction site and to PHD2 in the Fe2OG dioxygenase domain (Figure 1).

In the absence of quercetin, the center-weighted HIF1 α - pVHL interaction was -699.4 kJ/mol with only 2 hydrogen bonds, while in the presence of quercetin, the weighted score was reduced to -728.1 kJ/mol with 6 hydrogen bonds. The center-weighted score for HIF1 α and FIH interaction in the absence of quercetin was -846.0 kJ/mol and reduced to -854.2 kJ/mol in the presence of quercetin. The number of hydrogen bonds was also reduced from 12 to 5. The weighted score of HIF1 α and PHD2 in the absence of quercetin was -649.6 kJ/mol, and it slightly reduced in the presence of quercetin to -650.5 kJ/mol. The number of hydrogen bonds was also reduced from 15 to 10 hydrogen bonds (Table 2).

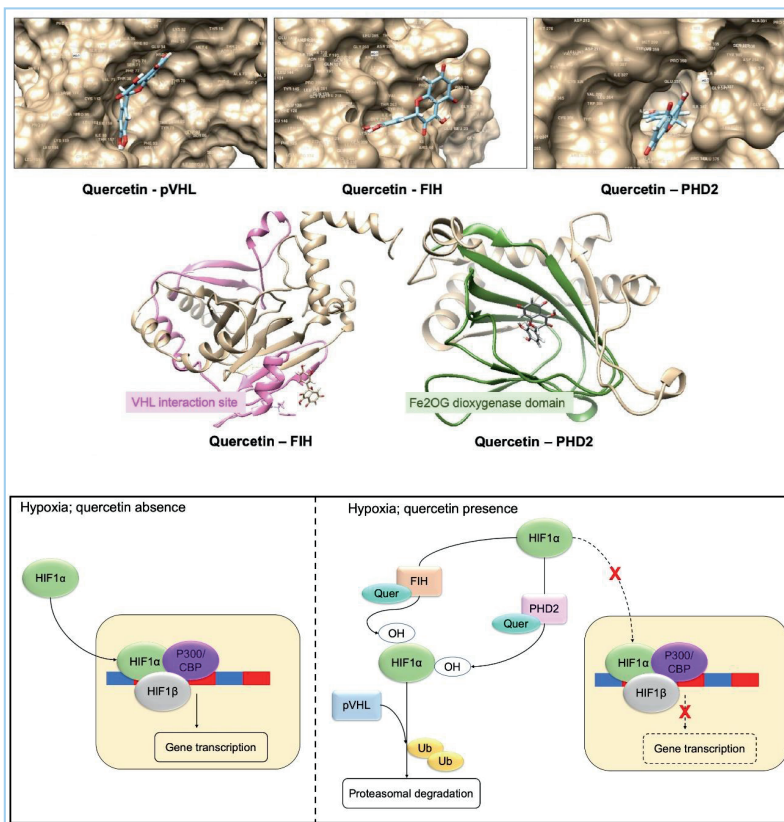


Figure 1. Binding site of quercetin on pVHL, FIH, and PHD2.

5. DISCUSSION

Protein HIF1 α is the component of the HIF-1 transcription factor. HIF-1 regulates many protein expressions involved in carcinogenesis, including vascular endothelial growth factor (VEGF), a critical factor in regulating the angiogenesis process. HIF-1 α overexpression is associated with treatment failure and increased mortality (16). Distinct enzymatic reactions provide the interface between oxygen and the HIF-1 α subunit: the hydroxylation of two prolyl residues (Pro402 and Pro564 in human HIF-1 α) in the oxygen-dependent degradation domain (ODDD) of the α -subunits (14, 15). This oxygen-dependent hydroxylation regulates the interaction of HIF1 α with the von Hippel-Lindau tumor suppressor protein (pVHL) (17).

Factor inhibiting HIF-1 (FIH) is a protein that binds to HIF-1 α and inhibits its transactivation function. FIH binds to pVHL, and the involvement of pVHL in association with FIH provides a possible mechanism for the modulation of HIF-1 α protein stabilization and transcriptional activation in response to changes in cellular O2 concentration (18).

Our study found that quercetin binds to FIH on the pVHL interaction site, suggesting this binding could promote FIH and pVHL interaction (Table 1, Figure 1).

Von Hippel-Lindau tumor suppressor protein pVHL is the recognition component of an E3 ubiquitin ligase complex that targets HIF- α for proteolysis by the ubiquitin-proteasome pathway (19). In this study, we found that quercetin could lower the binding energy between HIF1 α and pVHL, thus increasing the possibility of HIF1 α degradation by a ubiquitin-dependent pathway. In addition, the presence of quercetin also reduces the binding energy

between HIF1 α with FIH and PHD2, thus could increase the hydroxylation of HIF1 α by FIH and PHD2, leading to its proteasomal degradation or transactivation inhibition (Table 2, Figure 1). HIF-prolyl hydroxylases (PHDs) hydroxylate proline residues on HIF-1 α subunits leading to their destabilization by promoting ubiquitination by VHL ubiquitin ligase and subsequent proteasomal degradation. HIF-1 α transactivation is also repressed in an O₂-dependent way due to asparaginyl hydroxylation by FIH.

6. CONCLUSION

According to the silico analysis performed in this study, we found that quercetin could directly promote HIF1 α and pVHL interaction, thus increasing the degradation of HIF1 α by the ubiquitin-dependent pathway. In addition, quercetin could indirectly repressed HIF1 α expression by fostering interaction between HIF1 α and PHD2 and suppress HIF1 α transactivation by promoting its interaction with FIH. Further in vitro or in vivo study is required to support the result of this study.

- **Author's contribution:** F.R.P.D has a major role in the designing and data collection of this work. S.P.A.W had a part in data collection and article preparation, A.S.Y.H, L.M.L, V.L, L.I.L.A, and S.T.M have a part in manuscript validation.
- **Conflict of interest:** There are no conflicts of interest.
- **Financial support and sponsorship:** This work was funded by SA-TU-JRS Funding by Universitas Airlangga, Indonesia (grant number: 1301/UN3.15/PT/2021).

REFERENCES

1. Hyndman IJ. Review: the Contribution of both Nature and Nurture to Carcinogenesis and Progression in Solid Tumours. *Cancer Microenvironment* 2016; 9: 63-69.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144(5): 646-674.
3. Sharma H, Parihar L, Parihar P. Review on cancer and anticancerous properties of some medicinal plants. *J Med Plants Res*. 2011; 5: 1818-1835.
4. Teiten M, Gaascht F, Dicato M, Diederich M. Anticancer bioactivity of compounds from medicinal plants used in European medieval traditions. *Biochem Pharmacol*. 2013; 89: 1239-1247.
5. Dewi FRP, Ahmar RF, Alifiyah NI, Shoukat N Shoukat, Wahyuningsih SPA. The potential of *A. Muricata* Bioactive Compounds to Inhibit HIF-1 α Expression Via Disruption of Tyrosine Kinase Receptor Activity: an In Silico Study. *Acta Informmed* 2021; 29 (3): 176-181. doi: 10.5455/aim.2021.3.176-181.
6. Hollman PC, van Trijp JM, Buysman MN, van der Gaag MS, Mengelers MJ, de Vries JH, Katan MB. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett*. 1997; 418: 152-156.
7. Jeong JH, An JY, Kwon YT, Rhee JG, Lee, YJ. Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. *J Cell Biochem*. 2009; 106(1): 73-82.
8. Meneses AM, Wielockx. PHD2: from hypoxia regulation to disease progression. *Hypoxia (Auckl)*. 2016; 4: 53-67.
9. Zhang N, Fu Z, Linke S, Chinchin J, Gorman JJ, Visk D, Haddad GG, Poellinger L, Peet DJ, Powell F, Johnson RS. The asparaginyl hydroxylase FIH (Factor Inhibiting HIF) is an essential regulator of metabolism. *Cell Metab*. 2010; 11(5): 364-378.
10. Lee DH and Lee YJ. Quercetin suppresses hypoxia-induced accumulation of hypoxia-inducible factor-1alpha (HIF-1alpha) through inhibiting protein synthesis. *J Cell Biochem*. 2008; 105(2): 546-553.
11. Goel RK, Singh D, Lagunin A, Poroikov V. PASS-assisted exploration of new therapeutic potential of natural products. *Med Chem Res*. 2010; 20: 1509-1514.
12. Feixiong C, Weihua L, Yadi Z, Jie S, Zengrui W, Guixia V, Philip L, Yun T. admetSAR: a comprehensive source and free tool for evaluating chemical ADMET properties. *J Chem Inf Model*. 2012; 52(11): 3099-3105.
13. Desta IT, Porter KA, Xia B, Kozakov D, Vajda S. Performance and Its Limits in Rigid Body Protein-Protein Docking. *Structure*. 2020; 28(9):1071-1081.
14. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M et al. HIF-1 α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* 2001; 292: 464-468.
15. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ. Targeting of HIF-1 α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science*. 2001; 292: 468-472.
16. Semenza GL. Targeting HIF-1 for cancer therapy. *Nature reviews. Cancer*. 2003; 3(10): 721-732.
17. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999; 399: 271-275.
18. Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev*. 2001; 15(20): 2675-2686.
19. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE et al. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol*. 2000; 2: 423-427.