ORIGINAL PAPER

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

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ABSTRACT

Background: Cancer disease is a growing health problem in developing and developed countries. Hypoxia-inducible factor-1a (HIF1α) is a transcription factor responsible for expressing several proteins involved in angiogenesis. Quercetin can suppress HIF1α expression due to the inhibition of protein synthesis. However, to date, the study exploring the potential of quercetin in repressing HIF1α 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 HIF1α 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 HIF1α expression inhibitor was 0.969. In the absence of quercetin, the center-weighted score of HIF1α - pVHL, HIF1α - FIH, and HIF1α - 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 HIF1α - pVHL, HIF1α - FIH, and HIF1α - PHD2 was reduced to -728.1 kJ/mol, -854.2 kJ/mol, and -650.5 kJ/mol, respectively. **Conclusion:** Quercetin could directly promote HIF1α and pVHL interaction, thus increasing the degradation of HIF1α by ubiquitin-dependent pathway.

Keywords: Cancer disease, HIF1α, 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). HIF1α protein is subject to degradation during normoxia by the ubiquitin-proteasome pathway mediated by pVHL.

The association of HIF1α 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 HIF1α (FIH). FIH hydroxylase one asparagine residue in the C-terminal transactivation domain (C-TAD) of HIF1α. In addition, hydroxylation of

HIF1α by FIH blocks the association of HIF-1 with its transcriptional co-activators CBP/p300, thus inhibiting the transcriptional of the downstream target of HIF1α (8, 9).

A previous study reported quercetin suppressed the hypoxia-inducible factor-1α (HIF1α) accumulation during hypoxia in human prostate cancer LNCaP, colon cancer CX-1, and breast cancer SkBr3 cells. A previous study reported that suppression of HIF1α 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 HIF1α 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 HIF1α through its degradation mechanism pathway.

2. OBJECTIVE

The aim of this study was to investigate the potential of quercetin in regulating HIF1α 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://lmmd.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 HIF1α

Obtaining 3D structure of HIF1α, pVHL, FIH, and PHD2

The 3D structure of HIF1α (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)
HIF1α–pVHL	0	116	Center: -699.4 Lowest: -817.3	Hydrogen (2)	Ser343-Gln96; Arg306-Tyr112.
HIF1α–pVHL, quercetin	0	128	Center: -728.1 Lowest: -875.0	Hydrogen (6)	Glu277-Arg113; Tyr276-Arg113; Asp283-Arg205; Leu282-Arg205; His286-Arg205; Gly312-Asn131.
HIF1α–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.
HIF1α–FIH, quercetin	0	206	Center: -854.2 Lowest: -997.3	Hydrogen (5)	Asp285-Lys315; Arg306-Tyr348; Asp283-Tyr348; Arg311-Glu328; Lys251-Pro303.
HIF1α–PHD2	0	123	Center: -649.6 Lowest: -914.0	Hydrogen (15)	Tyr450-Glu318; Tyr450-Gln304; Ans448-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.
HIF1α–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 HIF1α 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), HIF1α 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 HIF1α expression by quercetin

HIF1α is the subunit of transcription factor HIF-1. Under the normoxic condition, HIF1α is localized at cytoplasmic and will be targeted by ubiquitinylation degradation. However, under low concentrations of oxygen, the degradation of HIF-1α is inhibited. The HIF1α 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-1α for proteolysis by the ubiquitin-proteasome pathway (14, 15).

In this study, we performed several docking analyses between HIF1α 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 HIF1α - 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 HIF1α 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 HIF1α 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).

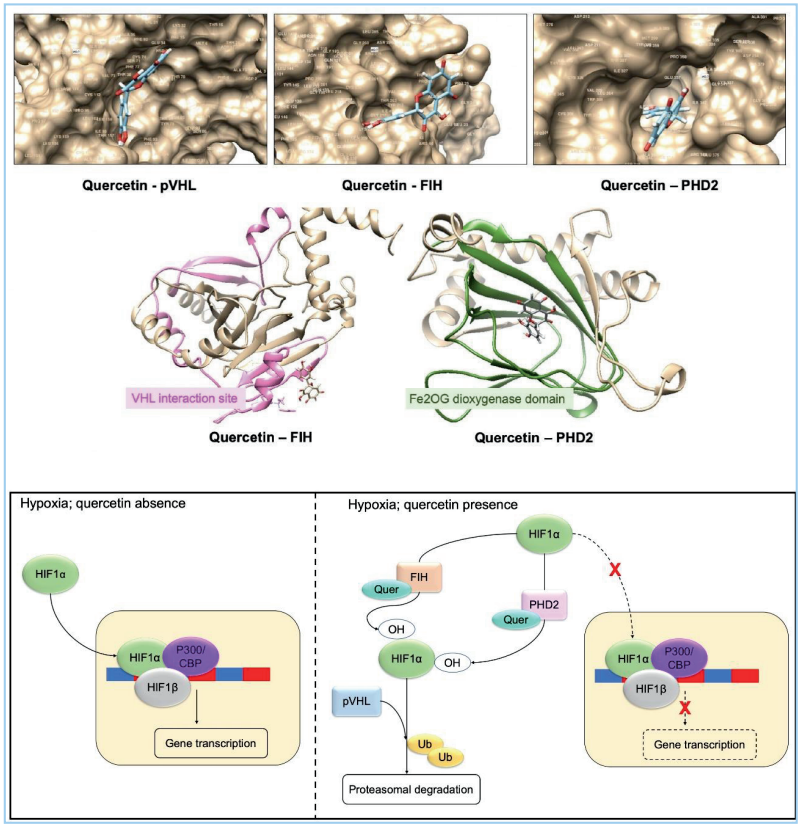

Figure 1. Binding site of quercetin on pVHL, FIH, and PHD2.

5. DISCUSSION

Protein HIF1α 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-1α overexpression is associated with treatment failure and increased mortality (16). Distinct enzymatic reactions provide the interface between oxygen and the HIF-1α subunit: the hydroxylation of two prolyl residues (Pro402 and Pro564 in human HIF-1α) in the oxygen-dependent degradation domain (ODDD) of the α-subunits (14, 15). This oxygen-dependent hydroxylation regulates the interaction of HIF1α with the von Hippel–Lindau tumor suppressor protein (pVHL) (17).

Factor inhibiting HIF-1 (FIH) is a protein that binds to HIF-1α 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-1α 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 HIF1α and pVHL, thus increasing the possibility of HIF1α degradation by a ubiquitin-dependent pathway. In addition, the presence of quercetin also reduces the binding energy

between HIF1α with FIH and PHD2, thus could increase the hydroxylation of HIF1α 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-1α subunits leading to their destabilization by promoting ubiquitination by VHL ubiquitin ligase and subsequent proteasomal degradation. HIF-1α 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 HIF1α and pVHL interaction, thus increasing the degradation of HIF1α by the ubiquitin-dependent pathway. In addition, quercetin could indirectly repressed HIF1α expression by fostering interaction between HIF1α and PHD2 and suppress HIF1α 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.
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