Pirin, a Multifunction Protein with Quercetinase Activity and Involvement in Transcription Regulation

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Manuscript submitted June 10, 2022; accepted September 7, 2022.
doi: 10.17706/ijbbb.2022.12.4.85-92

Abstract: Pirin is a highly conserved protein found in various organisms from bacteria to human. Although structurally it belongs to the widespread cupin family, we have limited knowledge of its function and mechanism. Recent research identified that Pirin can catalyze quercetin through quercetin 2,3-dioxygenase reaction and act as transcriptional co-regulator. To further investigate these functions, the relationship between Pirin and cancer development has been studied, identifying Pirin as a potential therapeutic target of cancer, particularly in melanomas, cervical cancer and squamous cell lung carcinomas. This review presents accumulating discovery in Pirin's function and discusses possible direction for further studies.

Key words: Applied computing, life and medical sciences, systems biology.

1. Introduction

Pirin is highly conserved in mammals, plants, fungi, and prokaryotes, classified as a member of cupin superfamily based on the similarity of protein sequence and structure [1]. Even though proteins in the cupin family have diverse functions, these proteins are classified into a family based on two structure features: a beta-barrel structure and two prominent sequence motifs: PGX5HXHX3,4-EX6G and GX5PXGX2HX3N [2, 3, 4]. Proteins in cupin family display diverse enzymatic and non-enzymatic functions. These biological functions are related to the two motifs' ability to bind metal ions, including nickel, iron, copper, zinc, manganese, and cadmium [5]. As a member of cupin superfamily, Pirin is also proven to have both enzymatic and non-enzymatic functions. For its enzymatic functions, Pang et al. first suggested that Pirin may have potential enzymatic activity, and later was proven by Adams and Jia that Pirin has quercetinase activity, more specifically quercetin 2,3-dioxygenase activity [6], [7]. For its non-enzymatic functions, Wendler et al. demonstrated that Pirin plays a role as a transcriptional co-regulator, assembling nuclear factor kappa B (NF-κB) with other factors [1]. Focusing on this pathway, researchers discovered the association between Pirin and cancer development [8]. In light of the association between Pirin and cancer, such as lung and cervical cancer, researchers have also developed probes based on Proteolysis targeting chimeras (PROTACs), a type of heterobifunctional molecules that degrade target proteins with E3 ligases, to degrade Pirin in order to application in cancer therapies [9].

2. Pirin's Structure

Pirin, with dot-like subnuclear structure, is a highly conserved 32 kDa protein consisted of 290 amino acids [1]. In human body, it is highly expressed in muscle and cardiac tissues. Even though pirin is found in all

organs, liver and heart have the highest level of pirin, while brain and pancreas have the lowest expressed level of pirin. In 1997, by cloning the PIR gene, Wendler et al. first expressed the protein from an in vitro model. They solved the structure of pirin which contains two beta-barrel domains, two signature cupin family motifs, a single Fe^{2+} (affords the protein with greater stability) in N-terminal domain and an alpha helix in C terminal domain. Through these characteristics, Wendler et al. classified pirin as a member of cupin family. Interestingly, the single Fe^{2+} allows the protein to gain greater stability, and C-terminal domain does not have metal-binding site typically found in other cupin proteins.

3. Pirin's Biological Function

3.1. Quercetinase Activity

3.1.1. Quercetin

Quercetin is an antioxidant flavonoid, found in onions, berries, broccoli, cherries, grapes, and citrus fruits [10]. Antioxidant can inhibit oxidation to protect cells from free radicals, unstable molecules produced from body [11]. Flavonoid is a phenolic substance, mostly found in fruit and vegetables. It can decrease the risk of cardiovascular diseases, metabolic disorders, and some types of cancer [12]. These amazing effects are due to flavonoid's ability of reducing the level of oxidative stress by inhibiting the aggregation of low-density lipoprotein oxidation and platelet and dilating blood vessels [13]. Radicals are accumulating when body are coping with environmental and other pressures, causing widespread damage to human body, which finally leads to disease such as cancer, Alzheimer's, cardiovascular disease, and renal diseases [14]. Flavonoid, with excellent oxidation resistance properties, plays a vital role in pharmaceutical industry as an alternative source of medicine for the diseases associated with oxidative stress. As an antioxidant flavonoid, or more specifically flavonol, querctin can protect tissues from various drug toxicities.

3.1.2. Quercetinase activity

Pirin has been shown to have quercetinase activity [7]. Pirin has similar folding structure to quercetin 2,3-dioxygenase. Quercetin 2,3-dioxygenase's N-terminal domain can be superimposed on Pirin's N-terminal domain, with r.m.s. difference of 1.5 Å for 84 equivalent residues. Furthermore, quercetin Cu-binding site in 2,3-dioxygenase is structurally similar to Pirin's metal binding site. Both quercetin 2,3-dioxygenase and Pirin use quercetin as substrate, and release CO at the end of enzymatic reaction (the reaction process of quercetin degradation has shown in the Fig. 1). Additionally, quercetin 2,3-dioxygenase's inhibitors can inhibit Pirin's enzymatic activity.

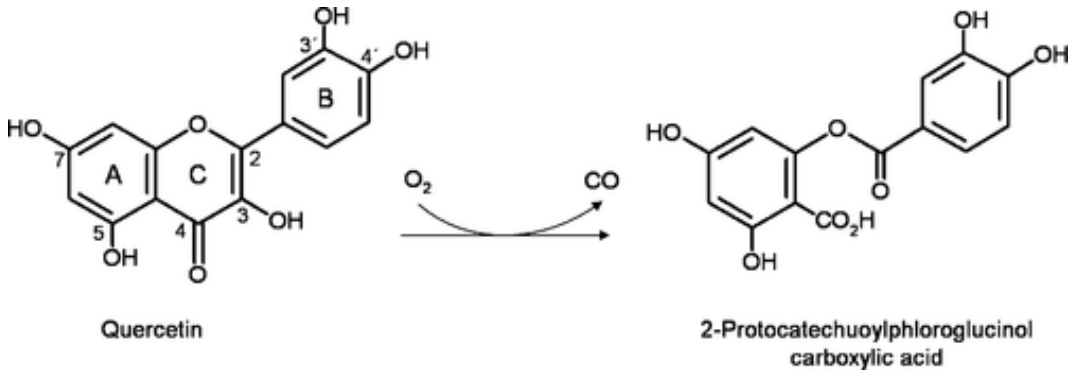

Fig. 1. Reaction process of quercetin degradation catalyzed by quercetinase (quercetin 2,3-dioxygenase).[15].

3.2. Transcriptional Co-regulator

Another function of Pirin is its role as a transcriptional co-regulator. In cell, Pirin first binds with nuclear factor I/CCAAAT box transcription factor (NF-I). Then it recruits and interacts to B-cell lymphoma 3-encoded protein (BCL-3), forming a complex with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [1], [16], [17]. NF-κB is a transcriptional factor that controls DNA transcription, cytokine production, and cell apoptosis, playing a key role in immune response, inflammation and oxidative stress [18]-[20]. Researchers discovered that Fe^{2+} bound in N-terminal domain of Pirin changes to Fe^{3+} under oxidating situation. As a result, Pirin is structurally altered, switching from inactive form to active form [21]. In the active form, Pirin has limited conformational flexibility and electrostatic complementarity, helping NF-κB to bind specific kB gene [22]. The discovery indicates that Fe ions are a reversible functional switch which allows NF-κB to respond alternation of the redox level in cell nucleus.

4. Implications of Pirin's Function

4.1. Quercetinase Activity

Quercetin can inhibit the expansion, or more specifically replication of poliovirus in cells. Nevertheless, overexpression of Pirin in cell decreases the level of quercetin's inhibition toward poliovirus. Neznanov and his team found that in HeLa cells, poliovirus has stronger resistant toward quercetin's inhibition on its replication than normal kidney human epithelial (NKE) or HEK293 cells. The reason is that HeLa cells have higher level of Pirin than NKE or HEK293 cells. On the other hand, poliovirus is more sensitive to quercetin's inhibition when siRNA induces suppression of Pirin levels. The mechanism behinds this phenomenon is Pirin's querctinase activity. This ability enables Pirin to degrade quercetin, meaning that the decline of quercetin concentration in cells result in weaker inhibition of poliovirus [23].

4.2. Transcriptional Co-regulator

4.2.1. Lung cancer

Lung cancer is one of the most common cancers that has been diagnosed in both male and female populations all over the world [24]. Tobacco smoke (TS) produced during burning cigarettes is the main cause of the cancer [25]. Researchers have observed a significantly increasing level of Pirin in long-term smokers' airway epithelium [26]. This remarkable rising level of Pirin is also considered as acute oxidative stress caused by cigarette smoke. One study suggests that to respond to cigarette smoke, pirin overexpression may play a role in cell apoptosis [27]. One possible mechanism is by the interaction between NF-κB and Pirin, which induces the expression of pro-apoptotic gene in those cells damaged by cigarette smoke. Even though the induction of pro-apoptotic gene may cause the chronic bronchitis after epitheliums have experienced chronic inflammation, it may be able to prevent the DNA damage and subsequent mutation which may result in lung cancer on the other hand [28]. Another study demonstrates that Pirin and TS's level is closely associated with chronic obstructive pulmonary disease (COPD), which usually will develop into lung cancer later [29]. Additionally, particulate matter (PM), a type of potent oxidative agent from air pollution, is an important risk factor of respiratory diseases and lung cancer. PM is associated with human respiratory fibroblasts' overexpression of Pirin [30].

4.2.2. Cervical cancer

Cervical cancer is the top 4 cancer in the ranking of most common cancers in female [31]. Human papillomavirus (HPV) is believed as a necessary condition of developing cervical cancer [32]. Researchers discovered that Pirin is expressed in an HPV load-dependent manner in cervical cancer cells [33]. Silencing Pirin causes increasing level of E-cadherin, which usually restricts the migration of cervical cancer cells, and the decreasing content of Slug, vimentin, Zeb and Snail, which is crucial for cancer cells to expand [34]. This

discovery indicates that Pirin is involved in cell migration and epithelial–mesenchymal transition (EMT). Different from the situation that Pirin associates with BCL-3 and Slug in melanoma cells, Pirin does not interact with BCL-3 and Slug [35]. Instead, it induces EMT by decreasing E-cadherin content in cells. In addition, one study has proved that curcumin, a common type of food additives and antioxidants, is able to reduce Pirin level, which results in limiting EMT and cell migration in HPV-18 and breast cancer cells [36], [37]. The ability of restricting EMT in cancer cells bestows curcumin pharmaceutical value.

4.2.3. Skin cancer

Melanoma, a major type of skin cancer, is diagnosed approximately 100,000 times every year [38]. Researchers found that pirin is related to cell migration in cancer cells. High expression of Pirin is responsible for melanoma's development in human body. In melanoma cells, Triphenyl compound A (TphA) can disturb the binding between Pirin and BCL-3, further restricting cell migration [34]. The progress of melanoma can be reflected by the location of Pirin. Pirin is usually found in cell nucleus and cytoplasm, but as the cancer develops, large portion of Pirin locates in cytoplasm [39]. In mature nervus cells, the expression of Pirin is much lower than melanoma cells. If the PIR is silenced, metastatic melanocytes alter both shape and size, indicating the cancer cells is senescent [40]. Outside stimulus can also induce the melanoma. Ultraviolet is one of the most common risk factors, especially UV-A [41]. UV-A will induce NRF2 nucleus translocation and accumulation [42]. The reactive oxygen species (ROS) production it promotes can then activate NRF2 in melanoma cells [43].

4.2.4. Breast cancer

About 13% of women in the US will suffer from invasive breast cancer, and the death rate of breast cancer is higher than any other type of cancer, except lung cancer. Even men are facing the risk of getting breast cancer, with a risk of about 1 in 833 [44]. As of 2021, breast cancer has become the most common cancer around the world. There is a significant variation between metastasis patients and non- metastasis patients [45]. Even though there is no significant difference between normal breast cells and invasive breast samples, Pirin is highly correlated with positive axillary lymph nodes status, indicating the correlation between Pirin level and cancer cell metastasis [46]. In addition, silencing the PIR gene can lower cancer cell proliferation. Pirin does not affect breast cancer development directly. Instead, it functions by activating E2F1, a key cell cycle regulator in malignant tumors [47]. The binding of Pirin and E2F1 will promote cancer cells to transit from G1 to S phase [48].

5. Applications of Pirin's Transcription Co-regulation Function

Proteolysis targeting chimeras (PROTACs), first developed by Sakamoto's team, are heterobifunctional molecules [49]. It is able to induce protein degradation through proteasome quickly and selectively [50]. As the Fig. 2 shows, the structure of PORTACs is like a dumbbell. The bone structure of PROTACs contains two ligands, linked by specific linker. One ligand selectively binds to target protein, such as Pirin. The other one recruits an E3 ubiquitin ligase. PROTACs work through recruiting an E3 ligase so that the E3 ligase is able to ubiquitinates the target protein, reaching the goal of degrading the target protein [51].

PROTACs have several advantages over traditional drug therapy, functioning as inhibitors. For regular inhibitors, the mechanism of drug action is protein-activity inhibition. In order to achieve sufficient inhibition, they need high systemic exposure, which may bring toxic side effects. Besides toxic side effects, prolonged exposure may also cause drug resistance. Compare to inhibitors, PROTACs function by degrading target protein with high efficiency. They do not require prolonged exposure for functioning so the toxic side effect they cause is weaker than traditional drugs and target proteins do not develop resistance toward PROTACs. Additionally, PROTACs are able to deal with proteins of interest (POI) that are not amenable to inhibitors, with a lower amount required to achieve efficacy than inhibitors [52].

Nevertheless, PROTACs, as a novel technique, still have several problems waiting for solutions. PROTACs' efficacy heavily depends on POI and E3 ligase ligands. Ligands' binding strength, space orientation, and other factors will affect the efficacy. Linkers' length and chemical properties will also influence PROTACs [53]. In addition, since PROTACs cannot actively locate POI, off-target effects may occur, causing safety problems. Therefore, researchers need to increase the selectivity of PROTACs to protect normal cells, which requires more efforts and time [54].

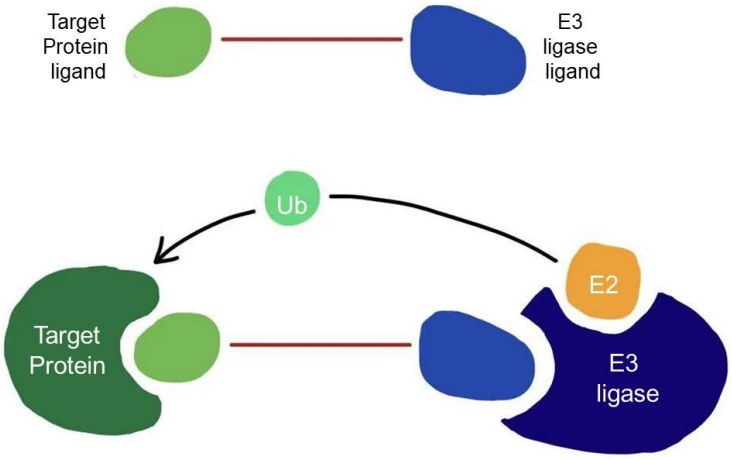

Fig. 2. Structure of protein degradation probe CCT367766.

A protein degradation probe (PDP) is a type of PROTAC. Chessum's team used PDP to develop PROTACs for Pirin functioning in SK-OV-3 human ovarian cancer cells. The first-generation PDP 3 did not reduce Pirin's level, and Chessum's team supposed that the issue could be low cell membrane flux. Therefore, in the second-generation PDP 10 they tried to decrease hydrogen bond donor count and tPSA in order to increase permeability. PDP 10 successfully declined the Pirin level in SK-OV-3, but it requires a high concentration of PDP 10 and the effect is poorly reproducible due to facile hydrolysis of the CRBN-targeting motif. The third-generation PDP 16, also called CCT367766, has higher permeability than the first two generations. Therefore, Pirin is completely degraded in a much shorter time with a lower concentration [9].

Even though there are more than 600 types of E3 ligases, only several of them are able to degrade proteins of interest. Von Hippel-Lindau (VHL) and cereblon (CRBN) ligands are the most common E3 ligands that are used in PROTACs. They have strong and selective binding affinity, and acceptable physical and chemical characteristics. Moreover, their binding modes' structural information is well-characterized [52]. Although VHL and CRBN are common E3 ligases, they are not able to degrade all types of target proteins. Therefore, developing different E3 ligase and their binding ligands is a crucial goal. Researchers now developed new E3 ligands, such as MDM2 ligands. Compared to traditional VHL and CRBN ligands, MDM2 ligands enable the PROTACs to have higher efficacy and can reduce the drug resistance of malignancies [55], [56].

6. Conclusion and Future Directions

Pirin is a conserved protein belonging to cupin superfamily. Currently we understand that the amount of Pirin in cells is closely related to cell migration, reflecting cancer development in some degree. However, the mechanism behind this connection is unclear. Based on the closed relationship between Pirin level and cancer, researchers have applied PROTACs to Pirin in order to decrease its level in cancer cell. Pirin is also shown to have quercetinase enzymatic activity. However, the related study on Pirin's quercetinase activity is limited. Because pirin is able to degrade quercetin and release CO which is a signal molecule that has implications in a wide range of physiological conditions. Since Pirin is a relative novel protein, we still have a long way to do

in gain full understanding of its function and mechanism in order to pave way for realizing therapeutic potentials.

Conflict of Interest

The author declares no conflict of interest.

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