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Review

Role of Pirin, an Oxidative Stress Sensor Protein, in Epithelial Carcinogenesis

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Simple Summary: Pirin is a protein which is detected at low levels in normal tissues. However, this is detected at high levels in multiple cancers, particularly in those with an epithelial origin such as melanomas, cervical cancer or squamous cell lung carcinomas. Basically, its role in cancer is related to the host response against factors causing oxidative stress, favoring cell migration and metastasis. Here we summarize the biological functions of Pirin related with its role in cancer, suggesting that Pirin is a potential therapeutic target.

Abstract: Pirin is an oxidative stress (OS) sensor belonging to the functionally diverse cupin superfamily of proteins. Pirin is a suggested quercetinase and transcriptional activator of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. Its biological role in cancer development remains as a novel area of study. This review shows accumulating evidence on the contribution of Pirin in epithelial cancers, signaling pathways involved, and as a suggested therapeutic target. Finally, we propose a model in which Pirin is upregulated by physical, chemical or biological factors involved in OS and cancer development.

Keywords: Pirin; cancer; epithelial

1. Introduction

The cupin superfamily of proteins is considered to be one of the most functionally diverse protein superfamilies. These proteins are characterized by their conserved β-barrel structure and two distinctive short signature sequence motifs: PGX5HXHX3,4-EX6G and GX5PXGX2HX3N which contain residues responsible for binding to metal ions. There is also a 15-50 amino acid sequence located between both motifs [1-4]. Interestingly, a range of metal ions bind to the cupin active site, including nickel, iron, manganese, copper, zinc, and cadmium, accounting for biological functions of cupin proteins [5]. They display diverse enzymatic functions such as dioxygenases, isomerases, oxalate oxidases and non-enzymatic activities such as auxin-binding, sucrose-binding, seed storage, and transcriptional factor, in Archaea, Eubacteria, and Eukaryota [1,6,7]. Although the focus was initially given to the physiological functions of cupin proteins, new insights reveal this superfamily may be involved in human diseases. For instance, two thiol dioxygenases cysteine dioxygenase (CDO) and 2-aminoethanethiol dioxygenase (ADO) have been associated with neurodegenerative

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disorders [8]. In addition, human σ1 receptor has been linked to diseases such as depression, drug addiction, neuropathic pain, as well as amyotrophic lateral sclerosis [9]. Nevertheless, the cupin protein activity in cancers is a promising area of research [8,10]. Pirin protein is a cupin superfamily member with a suggested role in cancer. Here, we reviewed the role of Pirin in epithelial carcinogenesis, its regulation, and its potential usefulness as a therapeutic target.

2. Pirin structure and biological functions

Pirin is a 32 kDa protein with 290 amino acids showing a dot-like nuclear localization, which is highly conserved among mammals, plants, fungi, and prokaryotes. In *homo sapiens*, Pirin transcripts are highly expressed in both muscle and cardiac tissues [11]. The Winaker´s group cloned the PIR gene for the first time and characterized the expressed protein from an *in vitro* model [11,12]. Pirin shares characteristics with cupin proteins such as two germin-like β-barrel domains, the two distinctive cupin family motifs, a single Fe2+ placed in its N-terminal domain, and a C-terminal domain that contains one α-helix and four β-strands (Figure 1). Curiously, this last domain does not include another metal-binding site found in other cupin proteins [3,11,12]. It was also described that the metal ion grants greater stabilization to Pirin's crystal structure and explains its biological functions [12]. Regarding its enzymatic function, the initial evidence showed that Pirin is functionally similar to quercetin 2,3-dioxygenase. In fact, both Pirin and quercetin 2,3-dioxygenase use quercetin, a flavonoid widely known for its antioxidant activity in human beings, as a substrate [13,14]. Additionally, carbon monoxide is released during the enzymatic reaction in both cases, and quercetin 2,3-dioxygenase inhibitors, are also able to inhibit Pirin activity, suggesting a quercetinase activity of Pirin [13]. In the same way, the Pirin bacteria ortholog $Pirin_{sm}$ interacts with pyruvate dehydrogenase E1 subunit, and consequently, inhibits and regulates the catabolism of pyruvate to acetyl-CoA [15].

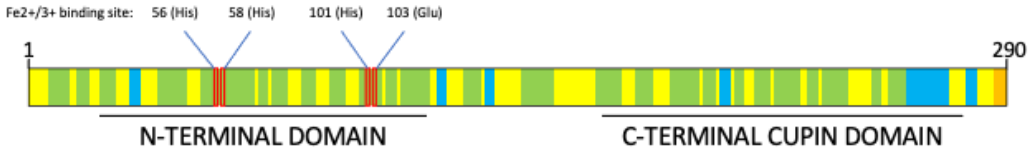

Figure 1. Pirin structure (*Homo sapiens*). Yellow: Protein Data Bank; Green: Beta strand; Blue: Helix; Orange: Turn. Red: Fe2+/3+ binding site. Data extracted from Uniprot Consortium [16]

The second function attributed to Pirin is acting as a transcriptional co-regulator. Initially, it was reported that Pirin interacts with the nuclear factor I/CCAAAT box transcription factor (NF-I) and binds to the hematologic oncogene B-cell lymphoma 3-encoded protein (BCL-3), forming complexes along with Nuclear Factor κB (NF-κB) p50 [11,17,18]. NF-κB is a ubiquitous transcriptional regulator considered central in immune response, apoptosis, inflammation, and oxidative stress (OS) [19-21]. More recently, it was described that under oxidizing conditions, Pirin metal structurally changes from Fe2+ (inactive form) to Fe3+ (active from) and functionally enhances the binding affinity to NF-κB p65, which suggests a previously unknown role of Pirin as an iron-depend OS modulator [22]. However, the Pirin Fe center does not represent its enzymatic active site, but functions as an allosteric control site. Meanwhile, the R-shaped surface loop area of Pirin is structurally modified depending on the metal oxidation state, and thus, the two resulting conformational states, active and inactive, are crucial for Pirin's ability to regulate NF-κB [22]. In addition, the Fe3+ form of Pirin shows restricted conformational space and electrostatic complementarity which are crucial for NF-kB binding [23,24]. Notably, these redox-mediator functions are also observed in plants and prokaryotes in which Pirin-like proteins regulate oxidative pathways and are closely related to cell death [25,26]. Additionally, Pirin is expressed at high levels in the kidney and spleen of transgenic cytosolic superoxide dismutase (Sod1)-deficient mice [27]. All the above findings are summarized in Table 1. In the Figure 1, an in-silico analysis showed that Pirin is functionally associated with WASF2, PSMA7, RAC1, NCKAP1, NCK1 and ABL2 proteins, which are involved in some tyrosine kinase pathways

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activation or lamellipodia formation. These associations, can explain at least in part, some of the biological functions attributed to Pirin.

Table 1. Biological functions of Pirin

Model	Function	Description	Reference
Human	Enzymatic	Quercetinase activity	[13]
Prokaryote	Co-Enzymatic	Inhibition of acetyl-CoA catabolism	[15]
Human	Transcriptional regulator	Interaction with NF-I/BCL-3/NF-κB p50	[11]
Human	Transcriptional regulator/Redox sensor	Binding to NF-κB p65 in oxidative conditions	[22]
Human	Transcriptional regulator	Fe active form favors its binding and regulation to NF-κB/DNA	[23,24]
Plants	Transcriptional regulator/Redox sensor	Regulation of oxidative pathways and cell death and redox sensor	[26]
Animal	Redox sensor	Activation in superoxide dismutase (Sod1)-deficient mice	[27]

[1] Abbreviations: NF-I: Nuclear Factor I; BCL-3: B-cell lymphoma 3-encoded protein; NF-κB: Nuclear factor kappa B; NRF2: Nuclear factor erythroid 2-related factor 2.

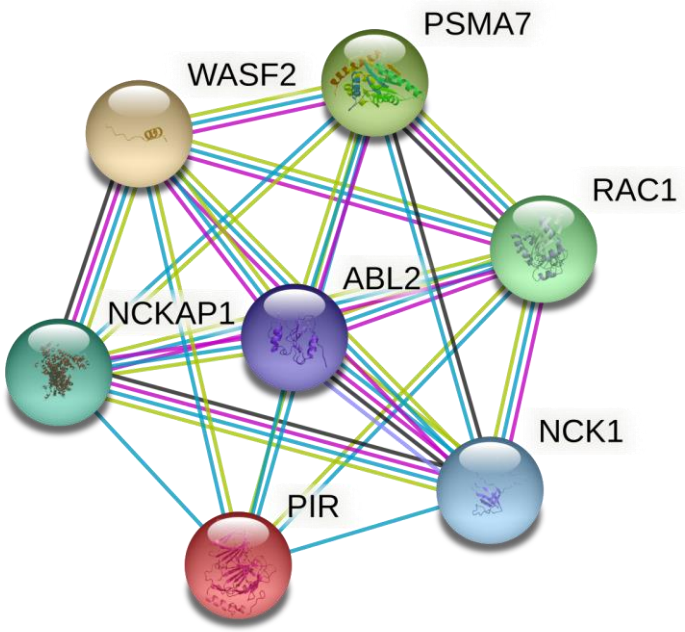

Figure 2. Pirin-protein interaction network. Filled nodes represent proteins with predicted 3D structures. Edges color, Purple: experimentally determined; light blue: association in curated databases; green: co-mentioned in PubMed abstracts (STRING V11.0, full STRING network, confidence 0.900) [28].

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3. Role of Pirin in cancer development

Recent investigations have been carried out on the contribution of Pirin in various types of cancer, including solid tumors [29], leukemia [30], and gliomas [31]. Additionally, Pirin has been reported as a molecular target of metastatic suppressors. However, the Pirin activity in cancer development remains a novel area of research [32]. The following paragraphs summarize the interaction of Pirin with carcinogenic agents and its potential role in epithelial carcinogenesis.

3.1. Lung cancer

Lung cancer is the most commonly diagnosed cancer in women and men worldwide, with tobacco smoke being the predominant cause [33,34]. It has been reported that Pirin levels are remarkably raised in the airway epithelium of chronic smokers [35]. Likewise, cigarette smoke extracts upregulate Pirin levels in human bronchial epithelial cells in a dose-dependent manner [36]. Such Pirin upregulation induces apoptosis of epithelial cells which may be explained by an interaction between Pirin and NF-κB [36]. Another study showed increased Pirin levels in human small airway epithelial cells exposed to TS, suggesting a potential role of Pirin in TS-associated injury [37]. Other studies demonstrated that Pirin is upregulated in bronchial epithelial cells exposed to cigarette smoke extracts and is accompanied by ferritin gene upregulation, a well-known marker of ferroptosis [38]. Furthermore, PIR has been identified as a novel NRF2-modulated gene in the small airway epithelium of healthy smokers [39]. NRF2, an oxidant responding transcription factor highly active in lung cancer cell lines [40,41], can bind to the PIR promoter which contains four functional antioxidant response elements (ARE) [39,42]. This association between NRF2 and PIR may explain a possible OS-regulation in the smoke-exposed airway epithelial cells [39]. Additionally, increased Pirin expression is found in alveolar macrophages of mice exposed to TS for 1 to 3 months, suggesting that Pirin may also be involved in macrophage activation [43]. Of note, Pirin levels shows a strong correlation with smoking as well as chronic obstructive pulmonary disease (COPD) [44], which frequently progresses to lung cancer. Moreover, particulate matter in outdoor air pollution, considered to be a potent oxidative agent and a significant risk factor for respiratory diseases and lung cancer [45], is associated with Pirin overexpression in human respiratory fibroblasts [44]. Additionally, telomerase RNA component (TERC) encoding gene, up-regulated in non-small cell lung carcinoma (NSCLCs) [46], was found to be regulated by Pirin [47]. Taken together, the above findings suggest a potential role of Pirin in lung cancer.

3.2. Cervical cancer

Cervical cancer is the fourth most prevalent cancer among women, reaching a total of 311 000 deaths in 2018 [48]. The persistent infection of high-risk human papillomavirus (HPV) is considered a necessary condition for cervical cancer development [49]. We demonstrated for the first time that Pirin is expressed in an HPV load dependent-manner in cervical cancer cells [50]. Additionally, it was demonstrated that PIR knockdown increases E-cadherin levels and reduces Slug, Zeb and Snail in cervical cancer cells, suggesting its contribution to epithelial-mesenchymal transition (EMT) and cell migration [50]. However, there is an interaction between Pirin, BLC-3, and Slug in melanoma cells, whereas in cervical cancer cells Pirin induces EMT by decreasing E-cadherin expression independently of Bcl3-Slug signaling [51,52]. Interestingly, we found that Pirin levels are increased by HPV16 E6 and E7 oncoproteins in infected cervical cancer cells, unlike HPV-negative cells [50]. Moreover, curcumin, a common food additive and well-known antioxidant agent [53,54], has been reported to interfere with EMT in HeLa cells (HPV-18) and breast cancer cell lines [55-57]. Notably, we demonstrated that curcumin decreases Pirin levels as well as reduces EMT and cell migration suggesting a novel Pirin-dependent mechanism wherein curcumin rescues cervical cancer cells from EMT [58].

It has been shown that basal Pirin expression is strongly dependent on NFR2, due to its crucial interaction with an ARE site located in a short region downstream of the transcription start site (TSS) [59]. Considering that Pirin is a NF-κB activator, we hypothesize that Pirin may act as a mediator

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between NRF2 and NF-κB in cervical cancer cells [59]. The Figure 1 shows immunohistochemically detected Pirin in cervical carcinomas.

3.3. Skin cancer

Skin cancer is the most common cancer in the United States when non-melanomas carcinomas are included in the registries [60], and approximately 100,000 new cases of melanomas are diagnosed per year [61]. In this epithelial cancer, a relevant role in cell migration and progression was identified for Pirin [51,62]. Miyasaki et al. identified high expression of Pirin in melanoma cell lines. In addition to this discovery, they found that a small molecule named Triphenyl compound A (TPh A), blocks the interaction between Pirin and BCL-3, in turn inhibiting cell migration [51]. It was also established that Pirin localizes in the nucleus or cytoplasm, depending on the stage of melanoma progression. In fact, a significant proportion of cytoplasmatic Pirin was found in metastatic melanoma cells compared to primary melanoma cells, suggesting that this pattern of Pirin localization may represent a cancer progression biomarker [62]. Moreover, the same group identified that Pirin is barely expressed in mature nevus samples compared to the high levels found in primary and metastatic melanomas. By inducing PIR knock down they also found that metastatic melanoma cells change both morphology and size, which was compatible with a senescent phenotype. Altogether, these results indicate that Pirin contributes to negatively controlled cellular senescence in melanomas [63].

It has been extensively reported that ultraviolet (UV) radiation is the major risk factor for melanomas [64]. It is important to note that UV radiation, specifically UV-A, induces NRF2 nuclear translocation and accumulation and may activate NRF2-controlled proteins in human skin cells [65]. Moreover, UV-A radiation promotes reactive oxygen species (ROS) production and subsequently triggers the activation of NRF2 in melanocytes [66]. Even though the mentioned findings consider Pirin regulator-NRF2 signaling pathways, there are still no studies that specifically assess the effect of UV radiation in Pirin levels. As previously stated, Pirin was recently identified as a potential target in antitumor therapies. In miR-155-overexpressing melanocytic cancer cells (CL16-miR-155), Pirin shows less intensity stain than controls by IHC assays [67]. Since miR-155 overexpression may inhibit tumor dissemination, extravasation, and colonization, Pirin may mediate, whether directly or indirectly, metastasis development [67,68]. In addition to these findings, after treatment with Hsc025, a novel molecule that improves hepatic fibrosis-degree and stimulates wound healing, Pirin levels modestly increase in fibroblasts [69,70]. However, Pirin knock down significantly counteract the effect of Hsc025 on fibroblasts migration, suggesting that Pirin may be a key intermediary in this process [70]. More recently, another novel compound called bisamide (CCT251236) was identified as a potent ligand of Pirin [71]. It is important to note that these molecules do not directly interact; in contrast, bisamide amide groups and Pirin's Fe bind through a water-mediated interaction. Additionally, there is consistent evidence that bisamide modulates the expression of the transcription factor HSF1 and subsequently inhibits the migration of human melanoma cells WM266.4; however, Pirin participation remains unclear [71]. In light of these findings, it was recently demonstrated that both CCG-222740 and CCG-257081, which are compounds structurally similar to CCT251236 and possible inhibitors of metastatic and fibrotic signals, are also able to bind to Pirin [32]. Moreover, they show that such CCG compounds may disrupt Pirin expression, and subsequently interfere with MRTF/SRF/DNA signaling, which has been associated with melanoma metastasis [32].

3.4. Breast cancer

In 2018, breast cancer was the second most prevalent cancer and the second cause of death among women in the United States [33,72]. Several genes have been shown to alter their expression pattern in human breast tumors, and some of these may have a role in predicting clinical prognosis [73,74]. One of these candidates includes the potential participation of Pirin gene [75]. Based on a previous report in which Pirin expression significantly varied between metastasis patients and non-metastasis patients [75], Shubbar et al. showed that Pirin levels in normal breast cells do not differ from invasive breast cancer samples. Nevertheless, Pirin levels are highly correlated with positive

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axillary lymph nodes status, suggesting a connection between high Pirin levels and local metastasis [76]. On the other hand, PIR knockdown resulted in a noticeably reduced cell proliferation rate in breast cancer cells and a decreased xenograft tumor growth in mice [77]. In addition, Pirin mediates breast tumorigenesis by promoting E2F1 expression, a key cell cycle regulator that is abnormally active in malignant tumors [77-79]. In fact, Pirin binds to the 3′-terminal region of E2F1 promoter and subsequently facilitates G1 to S phase transition in breast cancer cells [77].

3.5. Head and neck and gastrointestinal cancers

Other epithelial cancers such as oral, pancreas, biliary tract, and colorectal carcinomas have also been also linked with changes in Pirin expression. Firstly, we showed that Pirin is overexpressed in oral cells expressing HPV16 E6 and E7 oncoproteins [50]. Additionally, PIR gene expression analysis in head and neck carcinomas showed a statistically significant increase in HPV positive cases (Figure 2). Furthermore, both EGFR/MEK/ERK and PI3K/AKT pathways may be involved in the activation of Pirin by HPV-E7 oncoprotein in oral cells [50] which is in line with a previous report in transformed rat fibroblasts [80]. More recently, we demonstrated that EGFR/PI3K/AKT1/Nrf2 signaling promotes Pirin-dependent canonical NF-κB activation, which, in turn, promote oral cells migration [81]. Altogether, this strongly suggests a novel carcinogenic mechanism in oral cancer cells in which Pirin plays a crucial role. Similar to the aforementioned findings in lung cells [39], Pirin is strictly regulated by NRF2 in pancreas cancer cells [82]. Moreover, high Pirin levels are associated with a reduced survival probability in cholangiocarcinoma patients, suggesting that Pirin is a plausible prognostic biomarker [83].

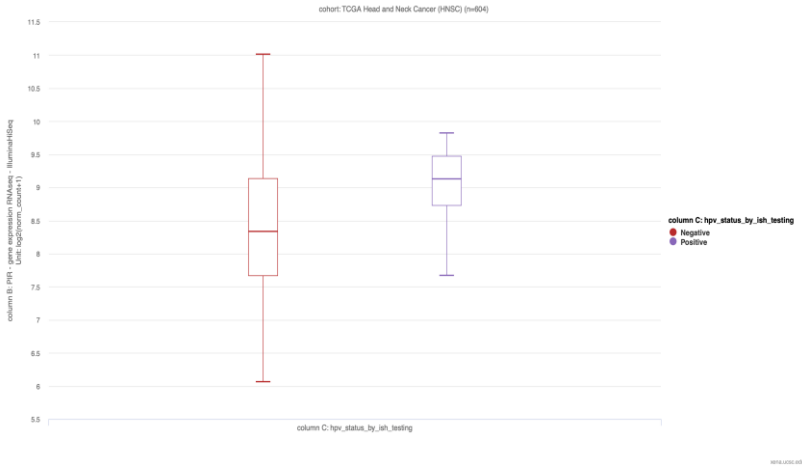

Figure 2. PIR expression in head and neck cancers (TCGA, N=604) stratified by human papillomavirus positive status. p=0.02028, Welch´s t-test. Data extracted from University of California, Santa Cruz (ena.ucsc.edu).

3.6. Non-epithelial cancers

Even though Pirin has been widely studied in epithelial cancers, other studies have proposed Pirin as a promising prognostic marker as well as an anti-proliferative therapeutic target of curcumin in neurological tumors [31,84]. Pirin is downregulated in acute myeloid leukemias, and indeed, its ablation may impair myeloid differentiation and maturation in both humans and mice [30]. Furthermore, Pirin is associated with contrasting high levels of peripheral white blood cells in B-precursor acute lymphoblastic leukemia, which entails a distinctive prognosis in leukemia patients [85,86]. In Figure 3, PIR expression in different epithelial and non-epithelial tumors extracted from TCGA database is shown.

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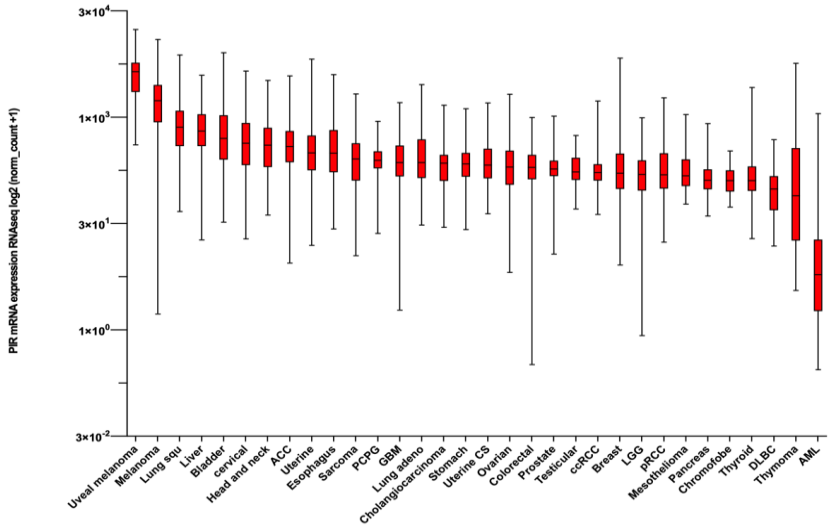

Figure 3. PIR expression in human cancer (TCGA, N=10071). $p< 0.0001$, Welch´s ANOVA test. Raw data were extracted from University of California, Santa Cruz (ena.ucsc.edu).

Table 2. Biological and chemical factors involved in Pirin regulation in human cancers[1]

Cancer	Factors	Regulation	Comments	Ref.
Lung	TS	Activation	Pirin levels are increased in airway epithelium of chronic smokers	[37]
	TS	Activation	Pirin overexpression occurs in a dose-dependent manner	[38,39]
	TS	Activation	Interaction with NF-κB resulting in a pro-apoptotic response	[38]
	TS	Activation	Pirin overexpression is accompanied by ferroptosis markers upregulation	[87]
	TS	Activation	Interaction with NRF2 in smoke-exposed airway epithelial cells	[29]
Cervical	E7 (HPV16)	Activation	Pirin regulates EMT and migration by interacting with NF-κB, Snail/Slug/ZEB proteins, and subsequently E-cadherin	[50]
	Curcumin	Suppression	Curcumin decreases Pirin expression, and consequently EMT and cell migration	[58]
Skin	TPh A	Suppression	Interferes the Pirin interaction with BCL-3, and consequently inhibits cell migration	[51]
	miR-155	Suppression	Pirin may mediate metastasis development	[68]

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	CCG	Suppression	Inhibition of carcinogenic signaling pathways	[32]
Oral	E7 (HPV16)	Activation	EGFR/MEK/ERK and PI3K/AKT pathways are involved in Pirin activation by HPV16 E7	[50]
	E7 (HPV16)	Activation	Upregulation of c-Rel and p65 through an interplay with Pirin, promotes cell migration and EMT	[81]

[1] Abbreviations: TS: Tobacco smoke; NF-κB: Nuclear factor kappa B; NRF2: Nuclear factor erythroid 2-related factor 2; BCL-3: B-cell lymphoma 3-encoded protein; EMT: epithelial–mesenchymal transition; HPV: Human papillomavirus; Slug: Zinc finger protein; ZEB: Zinc finger E-box-binding homeobox protein; EGFR: Epidermal growth factor receptor; MEK: Mitogen-activated protein kinase kinase; ERK: extracellular signal-regulated kinase; PI3K: Phosphoinositide 3-kinase; AKT: Protein kinase B.

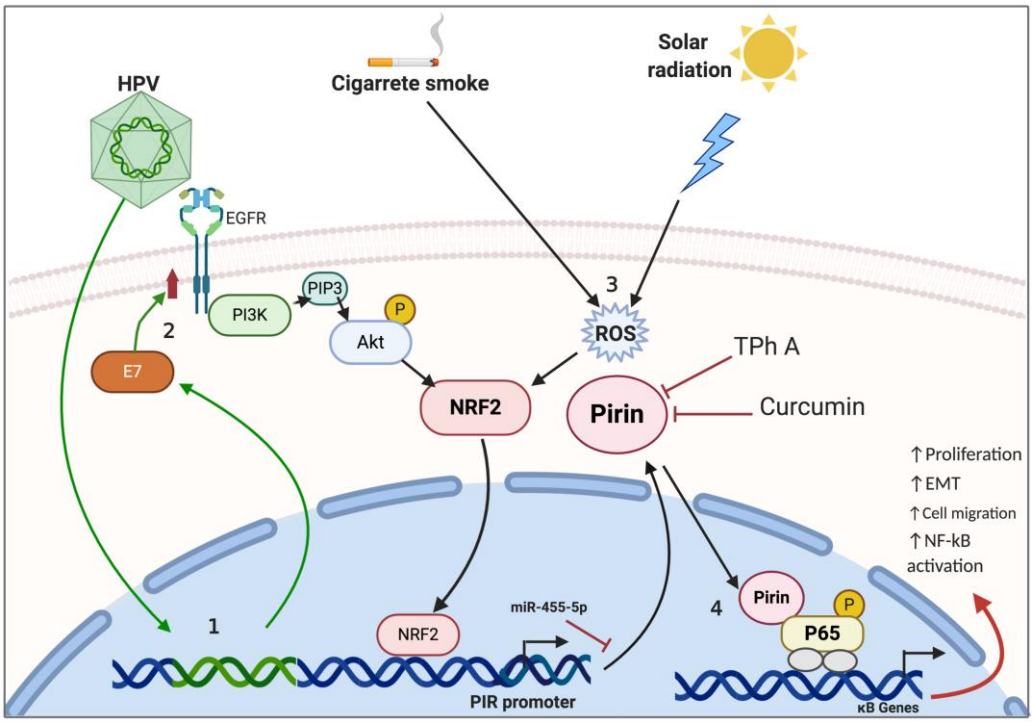

Figure 2. Model of Pirin-mediated tumor activation by viruses or environmental factors in epithelial cells. 1) HPV integrates into the host genome overexpressing viral oncogenes (ie. E7). 2) E7 promotes EGFR/PI3K/Akt activation and NRF2 recruitment into the PIR promoter leading to PIR expression. 3) Environmental factors such as cigarette smoke or UV radiation through ROS generation promote NRF2 recruitment into the PIR promoter. 4) Overexpressed Pirin (Fe3+ status) binds nuclear p65 promoting expression of κB genes. EGFR (Epidermal growth factor receptor), PI3K (Phosphoinositide 3-kinase), NRF2 (Nuclear factor erythroid 2-related factor 2), ROS (Reactive oxygen species), NF-κB (Nuclear factor kappa B), EMT (epithelial–mesenchymal transition).

Conclusions and Remarks

Pirin is an established OS sensor which is part of the functionally diverse cupin superfamily. Similar to other cupin proteins, Pirin shows enzymatic properties and acts as a nuclear transcriptional regulator. However, its potential oncogenic activity has been a growing topic of discussion in the past years. Recent findings have shown that Pirin plays a role in the development of cancer in epithelial

lung, skin, cervical, and oral tumors. Diverse factors (environmental, viral) promote ROS increase, in turn raising Pirin levels for activating NF-κB, cell proliferation, migration and EMT (Table 2 and Figure 2). This suggests a key participation of Pirin in cancer promotion and progression. Thus, this accumulating evidence provides auspicious signs of Pirin as an important therapeutic target in the years to come.

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