Mini-Review

Exploring molecular targets in cancer: Unveiling the anticancer potential of Paeoniflorin through a comprehensive analysis of diverse signaling pathways and recent advances

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Abstract

Tumors have posed significant threats to human health for over 250 years, emerging as the foremost cause of death. While chemotherapeutic drugs are effective in treating tumors, their side effects can sometimes be challenging to manage during therapy. Nonetheless, there is growing interest in exploring natural compounds as alternatives, which potentially achieve therapeutic outcomes comparable to conventional chemotherapeutics with fewer adverse effects. Paeoniflorin (PF), a monoterpene glycoside derived from the root of *Paeonia lactiflora*, has garnered significant attention lately due to its promising anti-cancer properties. This review offers an updated outline of the molecular mechanisms underlying PF's anti-tumor function, with a focus on its modulation of various signaling pathways. PF exerts its anti-tumor activity by regulating crucial cellular processes including apoptosis, angiogenesis, proliferation, and metastasis. We explored the multifaceted impact of PF while modulating through signaling pathways, encompassing nuclear factor kappa B, NOTCH, caspase cascade, transforming growth factor-β, NEDD4, P53/14-3-3, STAT 3, MAPK, MMP-9, and SKP2 signaling pathways, highlighting its versatility in targeting diverse malignancies. Furthermore, we discuss future research directions aimed at exploring innovative and targeted cancer therapies facilitated by PF.

Keywords: Paeoniflorin, Cancer, Apoptosis, Natural compound, Signaling pathway

1. INTRODUCTION

Human health has long been negatively impacted by tumors that have become a leading cause of mortality [1]. Consequently, the most recent Global Cancer Statistics for 2020 showed that 19.3 million new cancer cases were diagnosed, and about 10 million people died from cancer in 2020. Female breast cancer (11.7%) has topped lung cancer as the most frequent malignancy. Lung cancer (11.4%), colorectal cancer (10.0%), prostate cancer (7.3%), and stomach cancer (5.6%) are the next most frequently diagnosed cancers. With an expected 1.8 million fatalities (18%), lung cancer continued to be the primary cancer-related mortality factor, followed by colorectal cancer (9.4%), liver cancer (8.3%), stomach cancer (7.7%), and female breast cancer (6.9%). New instances of cancer are expected to be 28.4 million worldwide in 2040 [2]. The annual increase in cancer cases represents a significant concern and underscores the need for efficient prevention, early detection, and more effective treatment alternatives. As a result, many academics and practicing physicians are now focusing on developing proper medical strategies for the prevention and treatment of malignant tumors.

For a long time, cancer patients have had few treatment options available, which include surgery, radiation therapy, and chemotherapy, used either separately or in combination [3,4]. Combinational strategies combine several targeted or conventional chemotherapeutics such as taxanes and platinum compounds, which have been found to exert a synergistic effect [5]. Chemotherapy is still a popular treatment for a

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Received: 09 May 2024; Revision received: 14 June 2024; Accepted: 20 June 2024; Published:11 July 2024

How to cite this article: Jan K, Hassan N, James A, Hussain I, Rashid SM. Exploring molecular targets in cancer: Unveiling the anticancer potential of Paeoniflorin through a comprehensive analysis of diverse signaling pathways and recent advances. *J Biol Methods.* 2024;11(2):e99010014. DOI: 10.14440/jbm.2024.0003

wide array of malignancies at various stages. However, cancer cells frequently evolve drug resistance and stop responding to drug therapy, and exploring complementary and alternative medicines has been proven to be a formidable challenge [6]. Due to cumulative doses, a commonly used chemotherapeutic agent frequently causes cardiomyopathy and chronic heart failure, with prevalence ranging from 4% and 36% for cardiomyopathy and 0.2 – 8.7% for chronic heart failure [7,8]. On some occasions, it might be challenging to prevent the occurrence of adverse effects of a chemotherapeutic drug throughout treatment. However, medication combined with dietary supplements and phytochemicals opens a new avenue to achieving beneficial results with fewer side effects comparable to those of chemotherapeutic agents [9]. Different natural compounds such as taxanes [10], alkaloids [11], diarylheptanoids [12], flavonoids [13], polyphenols [14], and possess well-established anticancer properties. In addition to these natural agents, there are more natural substances having chemopreventive and chemotherapeutic potential.

Paeoniflorin (PF), one of the active constituents of *Paeonia lactiflora pall*, is a contemporary alternative Chinese medicine, known to have a number of desirable effects, including anti-inflammatory [15], anti-oxidative [16], anticancer [17], immunoregulatory [18], anti-depressant [19] properties, besides alleviating cardiac dysfunction [20], inhibiting platelet aggregation, dilating blood vessels, and reducing blood viscosity [21]. The anti-cancer effects of PF have reportedly been noted in a number of common malignant tumors such as lung [22], breast [23], gastric [24], bladder [25] pancreatic [26], colorectal [27], and other cancers through various mechanisms. PF primarily inhibits tumor growth and prevents metastasis and invasion of malignancies. However, there is still a dearth of comprehensive literature reviews that examine the molecular procedure behind the anti-tumor effects of PF. This review covers the most recent developments in the research regarding the anti-cancer characteristics of PF. We also discuss the molecular mechanisms of PF working against tumors and envisage the potential of using it as an anticancer medication in the future.

2. PF MEDIATING THROUGH NUCLEAR FACTOR KAPPA B (NF-KB) PATHWAY

NF-κB, a transcription factor, is considered to be a modulator of immune and inflammatory processes. The five-membered NF-κB family involves Rel A (P65), Rel B, C-Rel, P105/P50 (NF-κB1, and P100/P52 (NF-κB2). All these members share a highly conserved Rel homology domain that is important for dimerization, DNA binding, IKB interplay, and nuclear transcription [28]. In its dormant/inactive state, NF-κB is located in the cytosol, tangled with inhibitory protein IKB alpha. NF kappa is switched on by two important signaling pathways: canonical and non-canonical pathways [29]. Pro-inflammatory signals, such as cytokines, pathogen-associated molecular patterns, and danger-associated molecular patterns, are responsible for the upregulation of the canonical NF-κB signaling pathway [30,31].

The canonical pathway is activated through phosphorylation-dependent activation of the IKB kinase (IKKB) complex. Accordingly, IKB is degraded through site-specific phosphorylation of IKB alpha, releasing NF-κB, which migrates to the nucleus and transcribes the dependent gene. Non-canonical pathway responds only to a few TNFR signal receptors [30]. Genetic studies have confirmed that NIK is the key component of the non-canonical NF-κB signaling pathway [32]. The non-canonical pathway is activated through p100 processing [33]. NIK causes p100 phosphorylation at site-specific C-terminal serine residues through activation of IKK alpha, releasing Rel B; P52, which are translocated into the nucleus [28].

PF exerts anti-tumor [34], anti-oxidative [35], and anti-inflammatory effects by regulating the NF-κB pathway (Figure 1) [36]. Zhang *et al.* studied the anti-tumor activity of PF on human endometrial cancer cell line RL95-2 by detecting cell proliferation using the Cell Counting Kit 8 assay. During PF treatment, protein expressions were determined to observe alterations in various signaling pathways, such as MAPK, JNK, ERK, and NF-κB pathways in RL95-2 cells by Western blotting. The result showed that PF inhibited the proliferation of RL95-2 cells by activating MAPK and NF-κB pathways [34]. Wu *et al.* in 2008 found that PF played a protective role by down-regulating NF-κB expression in human gastric adenocarcinoma cell line SGC-7901 in a dose-dependent fashion, thereby triggering the death of gastric cancer cells induced by 5-fluorouracil [37]. PF was found

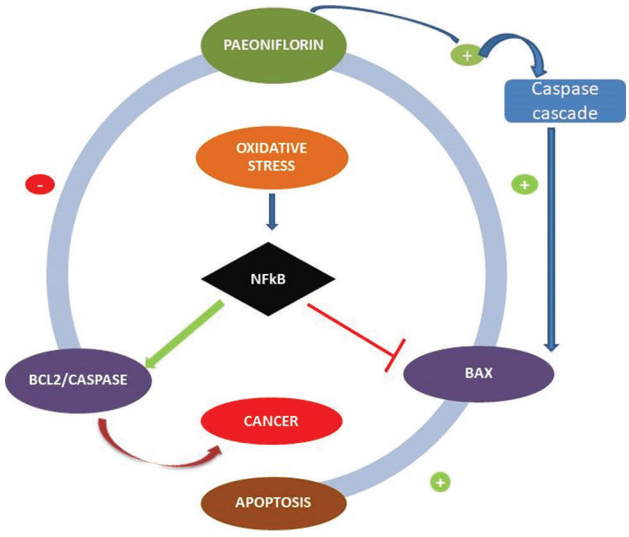

Figure 1. NF-κB activation in carcinogenesis and the protective role of Paeoniflorin in the regulation of signaling pathway

to disrupt the NF-κB signaling pathway to initiate caspase-3 activation, up-regulate Bax expression, and inhibit NF-κB p65 activation, ultimately triggering apoptosis of A549 lung adenocarcinoma cells by activating the NF-κB signaling pathway [22]. Similarly, PF increased caspase 3 expressions in HO8910 ovarian cancer cells, leading to a decrease in Bcl-2 and NF-κB P56, suggesting that PF exerted an anti-tumor effect on the ovarian cancer cell line [38]. Further research revealed that PF reduced the growth of human gastric cancer cell line SGC-7901/VCR and induced cell death by upregulating Bcl-2 expression and down-regulating NF-κB P65 through blocking the phosphorylation of IKBa, thereby impacting the proliferation of SGC-7901 gastric cancer cells and A375 melanoma cells [24]. According to the latest *in vitro* study, PF stimulated caspase-3 activity and suppressed the phosphorylation of p65 and IKBa, causing the hepatocellular cancer cell line Hep G2 to undergo apoptosis [39]. Furthermore, by increasing the ratio of Bax/Bcl-2, caspase-3 expression, PF promoted programmed cell death in hepatocellular carcinoma Hep G2 and SMMC-7721 cells [40] by suppressing the NF-κB pathway, upregulating pro-apoptotic genes, increasing caspase-3 and Bax activity and inhibiting anti-apoptotic gene Bcl-2 [35]. Similarly, PF exerted an anti-psoriatic effect by inhibiting the proliferation of HaCat cells through down-regulation of K17 and NF-κB signaling pathways in both *in vitro* and *in vivo* experiments. PF reduced the level of pro-inflammatory cytokines and K17, indicating that they could be the targets for the treatment of psoriasis [36]. The anti-inflammatory properties could exert a protective effect against TNBS-induced ulcerative colitis by suppressing the NF-κB signaling pathway [41]. PF also could protect amyloid beta (Aβ)-mediated neuro-inflammation in C6 glial cells by down-regulating the protein expression of NO synthase and cyclooxygenase-2 and blocking NF-κB signaling pathway through blocking phosphorylation of NF-κB and simultaneously elevating the protein levels of IKB alpha, Aβ-degrading enzyme (nephrilysin) [42]. Toll-like receptor 4 (TLR4) is one of the proteins in the TLR family which is a crucial regulator during the development and progression of cancer. Jiang *et al.*, in 2017, revealed that TLR4 was overexpressed in cervical cancer cell lines HeLa, Caski, and C33A by Western blotting and qRT-PCR [43]. Similarly, overexpression of TLR4 was indicative of a poor prognosis of glioblastoma [1]. Chuang *et al.* reported that PF therapy dose-dependently reduced the proliferation of glioblastoma cell lines through the Triad 3A-dependent ubiquitin-proteasome pathway [44]. Triad 3A is among the most significant E3 ligases that control TLR4 expression and plays a key role in accelerating TLR4 degradation by interacting with TLR4. PF increased the level of Triad 3A protein bound to TLR4 when used for the treatment of glioblastoma cell lines U87 and U251 in comparison to control cells. These results implied that Triad 3A could support the increased, ubiquitin-dependent degradation of TLR4 caused by PF [45]. In conclusion, PF works as a significant anti-tumor, anti-oxidative, and anti-inflammatory agent by modulating NF-κB pathway, promoting apoptosis, and inhibiting cancer cell proliferation across different types of cancer (Table 1).

3. PF MEDIATING THROUGH NOTCH 1 SIGNALING PATHWAY

NOTCH receptor is an evolutionarily-conserved single-pass transmembrane protein [46] with extracellular, transmembrane, and intracellular signaling domains, which are involved in different aspects of receptor activation [47,48]. The NOTCH signaling is initiated through the interaction between the NOTCH receptor present in one cell and ligands (delta such as 1-3 and jagged 1 and 2) present in the other neighboring cell [46]. During activation, the NOTCH receptor undergoes three proteolytic cleavages at sites S1, S2, and S3. Protein convertase causes S1 cleavage within the secretory system, allowing a processed heterodimeric form to be sent to the cell surface [49]. On ligand binding, protease ADAM10 (KUZ) performs S2 cleavage by removing the NOTCH extracellular domain, releasing membrane tethered version of the NOTCH intracellular domain. The later serves as a substrate for S3 cleavage. The transmembrane domain is then cleaved at site 3 by gamma-secretase complex, liberating NOTCH intracellular domain (NICD) [46], which migrates into the nucleus, forming a complex with CSL, mastermind-like (MAML), HAT P300, and other components of transcription [49]. Upon transcriptional activation, NICD is targeted for proteasome-mediated destruction by E3 ubiquitin ligases, such as Sel10, via phosphorylation by CDK8 to reduce the half-life of classical NOTCH signal and reset the cell in the preparation for the next signaling pulse [46].

NOTCH 1 pathway is crucial for apoptosis, growth, invasion, and metastasis of cancer [50,51]. Research has shown that PF has an anti-tumor effect on various malignancies, including breast cancer. Maraver *et al.* reported that PF significantly reduced the expansion of human breast cancer cell lines MDA-MB-231 and MCF-7 cells in a concentration-dependent manner by down-regulating the expression of the NOTCH 1 signaling pathway [52]. Similarly, Zhang *et al*., in 2017, reported PF exerted a depressing impact on the proliferation of the breast cancer cell line MCF-7 by down-regulating the expression of NOTCH 1 and its downstream target genes, such as *HES1*. MCF-7 cells were subjected to different PF concentrations at different intervals to measure cell proliferation, invasion, NOTCH1, and HES1 mRNA expressions. PF was found to suppress the growth of MCF-7 cells in both time- and dose-dependent fashion. The total number of cells was substantially lower in the PF

Table 1: Antitumor activities of PF mediated through different pathways

No.	Cancer	Exp. Material	Signaling pathways	References
1.	Endometrial cancer	RL95-2	NF-κB and MAPK Pathway	Zhang *et al.*, 2017 [34]
2.	Gastric adenocarcinoma	SGC-7901	NF-κB Pathway	Wu *et al.*, 2008 [37]
3.	Lung adenocarcinoma	A549	NF-κB and Caspase Cascade pathway	QI and Duan, 2015 [22]
4.	Ovarian Cancer	HO8910	Caspase cascade and NF-κB pathway	Gao *et al.*, 2019 [38]
5.	Melanoma	A375	NF-κB pathway	Fang *et al.*, 2012 [24]
6.	Gastric Cancer	SGC7901/VCR	NF-κB pathway	Fang *et al.*, 2012 [24]
7.	Hepatocellular carcinoma	HepG2 and SMMC-7721	Caspase Cascade, NF-κB, and SKP2 pathway	Xiang *et al.*, 2020; Liu *et al.*, 2020 [35,89]
8.	Cervical cancer	HeLa, Caski, and C33A	NF-κB and Caspase cascade pathway	Jiang *et al.*, 2017; Zhang and Zhang, 2011 [43,58]
9.	Glioblastoma	U87 U251	NF-κB and STAT3 Pathway	Wang *et al.*, 2018; Nie *et al.*, 2015 [45,79]
10.	Breast cancer	MDA-MB-231 and MCF-7	NOTCH 1 signaling pathway	Zhang *et al.*, 2016 [52]
11.	Colorectal cancer	HT29	STAT3 Pathway	Zheng *et al.*, 2014 [80]
12.	Bladder cancer	RY4	STAT3 pathway	Gandhi *et al.*, 2018 [81]

PF: Paeoniflorin; NF-κB: Nuclear factor kappa B

administration groups than in the untreated control group. The rate of cell invasion significantly decreased with increasing PF dosages, demonstrating a dose-dependent effect. Following treatment with 15 M and 30 M PF, there was a statistically significant reduction in NOTCH1 and HES1 mRNA expression levels in comparison to the untreated control group. Similar to the mRNA levels, NOTCH1 and HES1 protein levels showed that a rise in PF concentration was associated with a drop in NOTCH1 and HES1 protein expression levels. These findings imply that PF may prevent breast cancer cells from proliferation and migration by suppressing the NOTCH signaling pathway [53]. The mechanism underlying these actions might be more intricate. To establish a conceptual basis for the use of PF in the therapeutic cure of breast cancer, more research is warranted to clarify the precise mechanism by which PF mediates the reduction of cell proliferation and invasion in breast cancer cells.

4. PF REGULATING THROUGH CASPASE CASCADE PATHWAY

Apoptosis, a highly regulated process of cell death, is used to rid the body of useless, old, damaged, or contaminated cells. The primary agents of apoptosis are members of the caspase family of aspartate-specific cysteine proteases, that act in a cascade preserved through evolution [54]. These are produced as inactive zymogens within the cells, activating proteolytic activity during apoptosis. Caspases exert their effect by cleaving bonds at aspartic acid residues within proteins, which is accomplished by utilizing a nucleophilic cysteine in its active site [55]. There are two kinds of caspases: the inhibitor caspase encompassing caspase-2, -8, -9, and -10, and the effector caspase encompassing caspase-3, -6, and -7. Three caspase-associated pathways of apoptosis have been discovered in mammals: Extrinsic pathway or death receptor pathway driven by extracellular signaling molecules, intrinsic or apoptosome pathway mediated by mitochondria in response to certain death signals and cytotoxic lymphocyte-inhibited granzyme B pathway [56].

PF is proven to have antineoplastic effects on many tumor cell lines, including anti-inflammatory, and anti-tumor effects mediated through caspase cascade pathway (Figure 2). In osteosarcoma cell lines, PF leads to activation of cleaved caspase-3 and cleaved poly (ADP ribose) polymerase, besides stimulation of cell death. Moreover, the Bcl-2 X-associated protein and BH3 interacting domain death agonist are up-regulated and Bcl-XL, and Bcl-2 are down-regulated. In conclusion, PF inhibits osteosarcoma cell proliferation *in vitro* through mitochondria signaling pathway [57]. In HeLa cell lines, PF may lead to controlled cell death by inhibiting the anti-apoptotic gene Bcl-2 and up-regulating the pro-apoptotic genes Bax and Caspase-3 [58]. Treatment with PF reduced neuron loss and tributyltin chloride-induced damage in hypothalamic neurons in a dose-dependent way and the ratio of Bcl-2/Bax was elevated whereas the activation of caspase-3 was inhibited [59]. PF caused apoptosis of hepatocarcinoma cells via up-regulation of Bcl-2-associated X protein in HepG2 and SMMC-7721 cancer cell lines, up-regulated caspase-3 expression, and suppressed the phosphorylation of p65 and inhibitory kappa B in HepG2 cell lines [35].

5. PF REGULATING THROUGH TRANSFORMING GROWTH FACTOR-BETA (TGF-B/SMAD SIGNALING PATHWAY)

A large family of structurally similar pleiotropically-released cytokines includes the prototypical member TGF-β. TGF-β takes part in different cellular events like growth, differentiation, and apoptosis. Intracellular TGF-β signaling is regulated through SMAD proteins. SMAD family has three groups based on their respective functions: Receptor-regulated SMADS (R-SMADS), which include SMAD-1, -2, -3, -5, and -8, common SMADS (CO-SMADS),

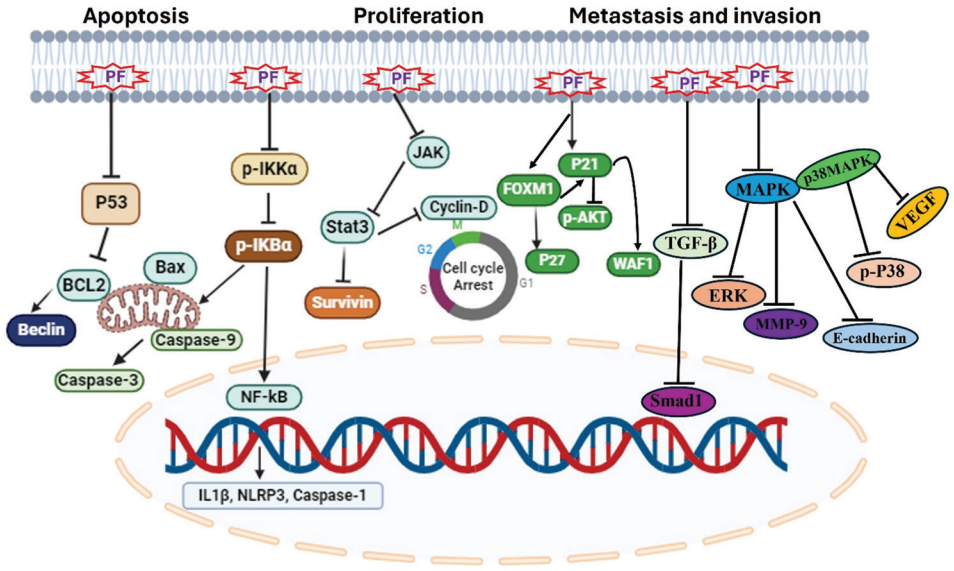

Figure 2. Mechanism of Paeoniflorin working on apoptosis, proliferation, metastasis, and invasion in cancer
PF: Paeoniflorin

involving SMAD-4, and inhibitory SMADS that consists of SMAD-6 and -7 [60]. TGF-β binds to its serine and threonine kinase receptors, the type II (TRII) and type I (TRI) receptors on the cell membrane, to trigger TGF-signaling. The receptor heterocomplex is created as a result of ligand binding, and TRII then phosphorylates the threonine and serine residues in the TTSGSGSG motif of TRI to activate TRI. The R-SMAD proteins SMAD2/3 for TGF-β and activin signaling and SMAD 1/5/8 for BMP signaling are recruited and phosphorylated by the active T-RI, which subsequently forms a heterocomplex with the Co-Smad SMAD4 (Make sure SMAD and Smad are used in the right way). Such Smad complexes are subsequently translocated into the nucleus, where they work with other co-factors to control the transcription of the target genes (Wrighton *et al.*, 2009). The transcription of the target genes is subsequently controlled by the Smad complexes in conjunction with other co-factors after being translocated into the nucleus [61].

PF has been shown to suppress TGF-β-induced epithelial mesenchymal transition (EMT), and TGF-β-induced activation of SMADS involved in EMT regulation, and PF is known to inhibit the migration and invasion of glioblastoma cells. The matrix metalloproteinase (MMPs) involved in cancer cell invasion was also found to be reduced [62]. In pulmonary fibrosis, PF inhibited TGF-mediated EMT at the early stages of alveolar epithelial cells, most likely by downregulating the production of the transcription factor Snail through a Smad-dependent mechanism that involves the upregulation of Smad7, an inhibitor of the TGF-1/SMADS pathway [63].

A study showed that the TGF-1/Smads signaling pathway, known to be involved in the pathogenesis of PCOS, was demonstrated to be suppressed by PF treatment. PF therapy reduced the expression of TGF, Smad2/3, and phosphorylated Smad2/3 proteins in the ovaries of PCOS rats apart from increasing the expression of Smad7, an inhibitor of the TGF-1/Smads pathway [64].

6. PF MEDIATING THROUGH NEDD4 SIGNALING PATHWAY

The earliest member of the family of E3 ubiquitin ligases is the neuronal precursor cell-expressed developmentally down-regulated 4 (Nedd4) [65]. Members of the HECT family of E3 ubiquitin-protein ligases are characterized by a C-terminal HECT domain that catalyzes the covalent binding of ubiquitin to substrate proteins as well as by N-terminal extensions with varying lengths and domain architecture that specify the substrate group of a particular HECT E3 [66]. NEDD4 family contains 9 proteins in humans, including NEDD4-1 (also known as NEDD4), NEDD4-2 (NEDD4L), ITCH, WWP1, WWP2, NEDL1 (HECW1), NEDL2 (HECW2), SMAD-specific E3 ubiquitin protein ligases (Smurf1), and Smurf222. Each of these nine NEDD4 family members comprises a C2 (Ca2+/lipid-binding) domain, 2-4 WW domains, and a HECT domain, and they are highly conserved E3s throughout evolutionary history. In malignancies, NEDD4-1 plays the twin roles of an oncogene and a tumor suppressor. The NEDD4-1 E3 ligase is suppressed by the enzyme-dead NEDD4-1-C867S mutant. The C2, WW, and HECT domains in NEDD4-1 interact with various substrates individually [67].

The substrate for NEDD4 identified include the epithelial sodium channel (ENaC), β2-adrenergic receptor (ADRB2), amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor, Notch, pAKT [21], insulin-like growth

factor-1 receptor (IGF-1R), vascular endothelial growth factor receptor-2 (VEGF-R2), Cbl-b, Deltex, epithelial growth factor receptor substrate 15 (EPS15), Spy1A, large tumor suppressor kinase 1 (LATS1), phosphatase and tensin homolog (PTEN), and mouse double minute 2 homolog (MDM2) [68]. PF exerted anti-cancer effects on nasopharyngeal carcinoma (NPC) cells by inducing apoptosis of NPC cells and PF treatment significantly decreased viability, colony formation, and migration of cancer cells. The study also revealed that NEDD4, a cellular protein, was down-regulated by PF, thereby producing its anti-cancer effects. According to the study, the PI3K/Akt signaling pathway was suppressed by PF, which, in turn, brought about the down-regulation of NEDD4 [69]. PF could cause ferroptosis, or iron death, of human glioma cells by modulating the NEDD4L/STAT3 pathway. With glioma cells, PF administration promoted ferroptosis and lowered the viability of glioma cells. The underlying process involved the NEDD4L inhibition, STAT3 activation, and subsequent induction of ferroptosis [70].

7. PF MEDIATING THROUGH P53/14-3-3 PATHWAY

P53, a transcriptional factor that has been well-studied, is essential for maintaining genetic integrity. It belongs to a larger gene family, along with the remarkably similar proteins p63 and p73 [71].

The p53 pathway, a crucial tumor-restricting mechanism, inhibits the transformation and development of cancer cells and is typically shut down by malignancies as they develop. However, only around 50% of them have p53-inactivating mutations. With the remaining 50% of the malignancies, alterations of the proteins that regulate the pathway reduce p53 activity [72]. Favorable modulators of the tumor suppressor p53, whose mutation is linked to several human cancers, are 14-3-3 proteins [73].

The tumor suppressor p53 is stabilized by the adaptor protein 14-3-3, which also increases the anti-tumor efficacy of the protein. According to Schumacher *et al.*, the p53 tetramer is stabilized by 14-3-3 binding to the C-terminus, which is crucial for the transcriptional activity of the p53 protein. The molecular processes behind this stabilization could be explained by the structure of the p53 C-terminus coupled to 14-3-3 [74]. PF leads to the suppression of tumor and cell growth in a dose-dependent manner by arresting the cell cycle at the G1 phase that is associated with DNA damage and up-regulation of P53/14-3-3 zeta in human colorectal carcinoma HT 29 cells [75].

8. PF REGULATING VIA STAT 3 SIGNALING PATHWAY

STAT3 is a transcriptional factor that was found to be a transducer of signals from the cell surface receptors to the nucleus. It is activated by the phosphorylation that leads to the formation of dimers eventually and stimulates the gene transcription by binding to the DNA [76]. STAT3 plays a key role in determining whether the immune response inhibits or promotes cancer growth. Activated STAT3 increased the growth and survival of tumor cells by promoting different pro-oncogenic inflammatory pathways like JAK, NF-κB pathways [77]. STAT3 is one of the suitable targets for cancer therapy. It regulates the function of mitochondria as well as the expression of genes via epigenetic mechanisms [78]. PF inhibits not only the activation of JAK2/STAT3 signaling but also the increased production of pro-inflammatory cytokines like MCP-1, TNF-Alpha, and IL-1B [79]. It induces ferroptosis in human glioma (a kind of fast-growing and aggressive brain tumor) cells by regulating the STAT3 pathway [79]. PF inhibits the cellular division of gastric carcinoma, lung cancer, and colorectal cancer in humans by increasing the expression of miRNA (small non-coding RNA sequence) [80]. It also inhibits bladder carcinoma growth by deactivating STAT3. Activation of STAT3 induces the proliferation of bladder cancer cells, resulting in their survival. However, treatment with PF via regulating the phosphorylation of STAT3 in RY4 cells (bladder cancer cells) lead to inhibition of cancer cell proliferation [81].

9. PF MODULATING THROUGH MITOGEN-ACTIVATED PROTEIN KINASE (MAPKS) PATHWAY

MAPKs are a family of protein kinases that play crucial roles in various cellular processes, including differentiation, proliferation, and cell death. The mammalian MAPK family has 3 sub-families: extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 mitogen-activated protein kinases (p38s). Each of the cascades activates a particular MAPK, that is, MAPK kinase kinase (MAPKKK) and MAPKK kinase (MAPKK). MAPKKK is activated when it interacts with GTPase which breaks GTP to GDP or when it is phosphorylated by protein kinases. MAPKKK activates the MAPKK by phosphorylation, which then activates the MAPK. MAPK phosphorylates many substrates in the nucleus and cytosol, changing the function of protein and expression of genes that perform a specific biological function.

The ERKS are responsible for cell growth and differentiation. The JNK is involved in the production of cytokines, apoptosis, and metabolism. The P38 family includes p38α, p38β, p38γ, and P38δ, which are involved in inflammation, apoptosis, differentiation of cells, and regulation of the cell cycle [82].

PF prevented p38 MAPK and JNK from being activated but increased the activation of ERK, and due to the inhibition of MAPK-mediated inflammatory response, PF could protect rats against ischemic injury [83]. PF prevented p38 phosphorylation and arrested the cell cycle in the S phase [84].

PF also played a role in cardiac protection by altering the MAPK signaling pathway [85]. Exposing the cells to the ionizing radiation stimulated the MAP kinase pathway and, as such, the irradiated cells produced damaging reactive oxygen species, which were protected by the PF which modulated the reactive oxygen species and MAP kinases (Figure 2).

10. PF MEDIATING THROUGH MMP-9 SIGNALING PATHWAY

MMPs are a family of enzymes that play a crucial role in regulating various physiological and pathological processes. They are involved in wound healing, tissue development, and the progression of multiple diseases, making them essential for maintaining tissue homeostasis and integrity. One of the members of MMPs is MMP-9, which is also called 92KDa type 4 collagenase or gelatinase B. MMP-9 disrupts the extracellular matrix protein and activates chemokines and cytokines to regulate the remodeling of tissue, the final phase of the healing process. The myocardium contains a large number of extracellular matrix proteins, such as collagens, and fibronectin, which are important for the functioning of the heart. MMPs cause the breakdown of the extracellular matrix by disrupting its structural elements. In humans, MMP-9 genes code for an enzyme called pre-pro-enzyme made up of 707 amino acids and it contains four domains: amino-terminal propeptide, carboxyl-terminal domain, catalytic domain, and signal peptide. Plasmin can activate certain MMPs, including MMP-9, and can degrade various components of the extracellular matrix, which is important for the development of embryos, reproduction, development of bones, and cell migration [86]. PF could alleviate post-operative pain by inhibiting MMPs, that is involved in the induction of inflammation with the release of inflammatory cytokines ("maturation of cytokines?" Are you sure the meaning is right?). It also induced the apoptosis of pancreatic cancer cells [87].

11. PF REGULATING THE S-PHASE KINASE-ASSOCIATED PROTEIN 2 [SKP2] PATHWAY

SKP2 pathway is an important regulating pathway that controls the cell cycle progression, specifically facilitating the transition from G1 to the S phase, and regulates apoptosis, cell proliferation, migration, and angiogenesis. SKP2 protein, also called p45, is a protein complex that is a component of the ubiquitin ligase complex, which plays a key role in the proteolysis of specific proteins involved in cell cycle regulation [88].

PF has anti-inflammatory, anti-oxidative, anti-platelet, and anti-tumor activity and can inhibit the survival of cancer cells by down-regulating the SKP2 in liver cancer cells [89]. The compound is effective against various types of cancers. SKP2, an oncoprotein contributing to oncogenesis can be inhibited by PF. The expression of SKP2 is limited by PF in the liver cancer cells, resulting in the suppression of cell survival, cell migration, and apoptosis induction. However, the over-expression of SKP2 abolished the anti-cancer activity of PF whereas the down-regulation of SKP2 enhances the PF activity [89]. In glioma cells, SKP2 expression is down-regulated after treatment with PF [90].

Some evidence showed that PF worked against cancer modulation through Fas/Fas, Hippo, and Fos-Jun-DNA pathways. For instance, in non-small-cell lung cancer cells, PF enhanced the apoptotic pathway mediated by the Fas/Fas ligand, causing cell cycle arrest and thereby suppressing cell growth [91]. Further investigation revealed that PF exerted a pro-apoptotic effect on human hepatoma cells by inhibiting the prostaglandin E receptor EP2. Anti-tumor effect of PF on gastric cancer cells via the hippo signaling pathway has also been documented [26].

PF exhibited a potent cytotoxic effect on HL-60 and other leukemia cell lines by inhibiting the formation of the Fos-Jun-DNA complex. In addition, PF upregulated the expression of HTRA3, a member of the highly-conserved stress-related serine protease HtrA family. This upregulation of HTRA3 effectively restrained the proliferation of pancreatic cancer cells, specifically Capan-1 and MIAPaCa-2 cells [92].

12. CONCLUSION

PF possesses anti-oxidative, anti-inflammatory anti-fibrotic, pro-apoptotic, and anti-angiogenic effects on tumors of various types or from different organs by regulating different pathways. The literature has established that PF exerted an anti-cancer effect by inhibiting NF-κB, NOTCH1, JAK2/STAT3, MAPK, SKP2, NEDD4, and TGF-Beta signaling pathways and activating caspase cascade, P53/14-3-3 signaling pathways, making it a promising candidate oncotherapeutic. Despite a great many investigations, further studies are needed to fully comprehend how PF works against tumors.

ACKNOWLEDGMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

The data that support the findings of this study are available on request from the corresponding author.

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