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REVIEW PAPER

# Recent Insights into the Roles of PEST-Containing Nuclear Protein

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## Abstract

PEST-containing nuclear protein (PCNP), a short-lived small nuclear protein with 178 amino acids, is a nuclear protein containing two PEST sequences. PCNP is highly expressed in several malignant tumors such as cervical cancer, rectal cancer, and lung cancer. It is also associated with cell cycle regulation and the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) and Wnt signaling pathways during tumor growth. The present article discuss how PCNP regulates the PI3K/AKT/mTOR and Wnt signaling pathways and related proteins, and the ubiquitination of PCNP regulates tumor cell cycle as well as the progress of the application of PCNP in the pathophysiology and treatment of colon cancer, human ovarian cancer, thyroid cancer, lung adenocarcinoma and oral squamous cell carcinoma. The main relevant articles were retrieved from PubMed, with keywords such as PEST-containing nuclear protein (PCNP), cancer (tumor), and signaling pathways as inclusion/exclusion criteria. Relevant references has been included and cited in the manuscript.

**Keywords** Cell cycle · Cancer · PCNP · PI3K · Wnt

## Introduction

The PEST protein domain contains extremely high levels of serine (S), glutamic acid (E), threonine (T), and proline (P), followed by histidine (H), lysine (K), or arginine (R) residues, which are mainly present in some short-lived proteins such as transcription factors. This class of nuclear proteins (NPs) are called PEST-NPs [1]. PEST-NPs participate in cancer metabolism, immune response and protein transcription and are, therefore, good targets for cancer therapy [2]. These proteins are also associated with functional proteins such as transcription factors, cyclins, proto-oncogene expression proteins, and other functional proteins. The PEST sequence is a protein hydrolytic enzyme recognition signal that can degrade residual proteins. Moreover, residual proteins can mediate the regulatory proteins of cell signaling pathways involved in cell proliferation, differentiation, stress, and apoptosis [3].

In 1986, Rechsteiner et al. proposed that PEST proteins can rapidly induce hydrolytic disruption because of the associated PEST domain [4, 5]. PEST proteins are expressed in many species and have diverse functions including participation in ubiquitin-mediated proteolysis, nucleocytoplasmic transport, stability of nuclear proteins, cell cycle regulation, and cyclic nucleotide signaling pathway. PEST-containing proteins interfere with the ubiquitin proteasome pathway,

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nuclear pore glycosylation, and hexosamine biosynthesis pathway at the molecular level. A new PEST containing nuclear protein was (PCNP) first reported in 2002 by Mori et al. [6]. In the protein hydrolysis catalyzed by the ubiquitin proteasome system, PEST proteins play a regulatory role in controlling cellular regulation. In addition, another nuclear protein containing PEST can ubiquitinate PCNP, called NIRF [7]. The domain of NIRF is similar to the characteristic domain of cell proliferation-related protein Np95/ICBP90 [8, 9]. NIRF is involved in cell cycle regulation, and the overexpression of it can cause G1 phase cell proliferation [10]. Both NIRF and Np95/ICBP90 [11, 12] are members of the NIRF family. In vitro and in vivo experiments have also shown an association between PCNP and NIRF [6]. On the basis of these findings, researchers have proposed that the nuclear protein PCNP acts as a substrate of NIRF and participates in the ubiquitination process of proteins (The involvement of nuclear protein PCNP as a substrate for NIRF in the ubiquitination process of proteins is shown in Fig. 1).

In 2020, the number of new cancer cases worldwide reached nearly 20 million. Leukemia, central nervous system tumors, and lymphoma were among the top three high incidence cancers in children between 2018 and 2020 [13].

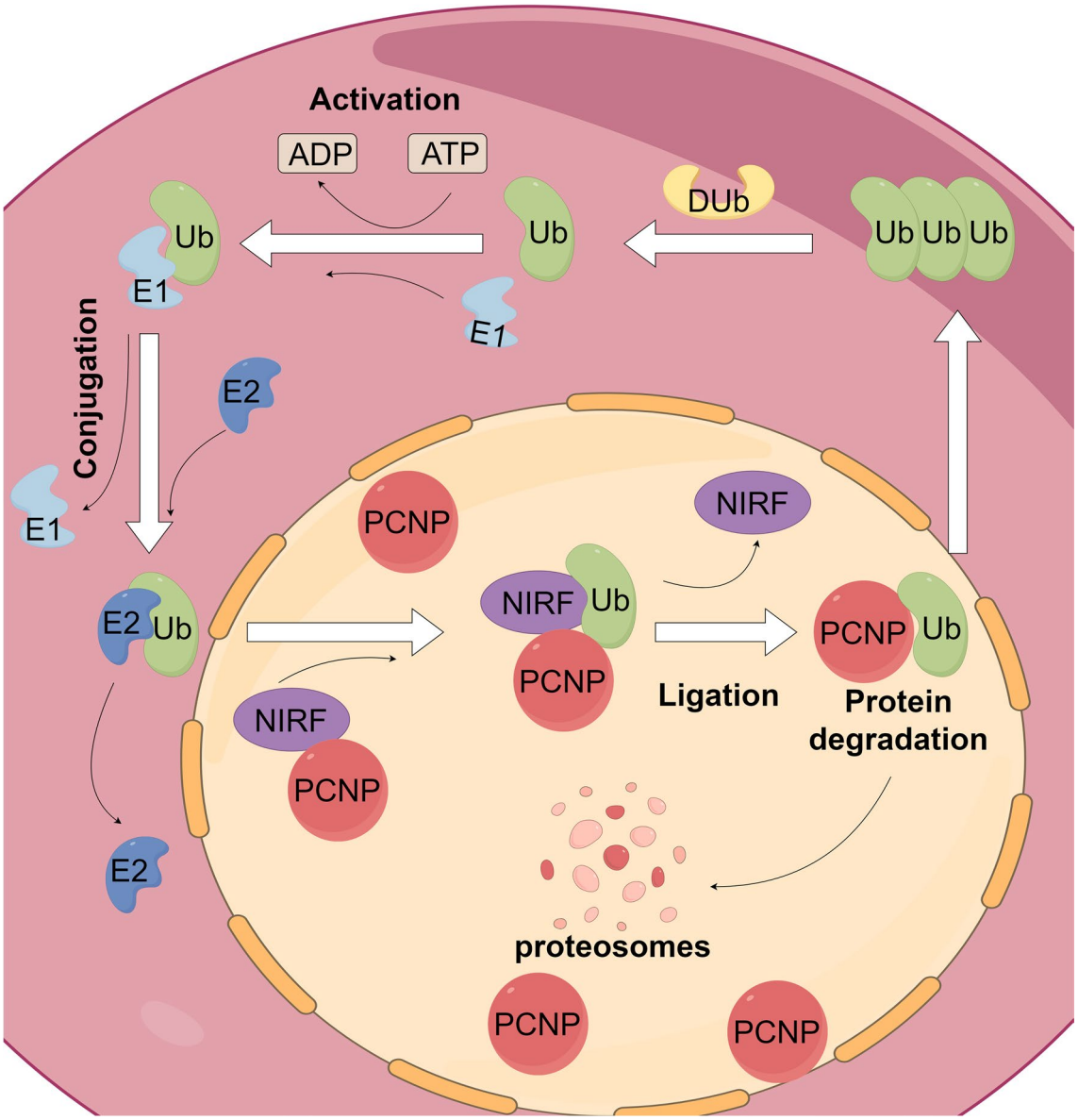


**Fig. 1** NIRF promotes ubiquitination of PCNP in nuclear cells: E1 and E2 ligases promote the binding and connection between NIRF and PCNP. The C-terminus of NIRF executes ubiquitin ligase function, acting as a substrate on PCNP and regulating proteasome activity (Created with Figdraw.com)

According to previous studies, PCNP in neuroblastoma [14] and human thyroid cancer can inhibit the occurrence of tumors, while its function in ovarian cancer [15] lung adenocarcinoma [16] and oral squamous cell carcinoma can promote the occurrence of tumors. PCNP, a short-lived small nuclear protein containing 178 amino acids, is a nuclear protein containing two PEST domain. It participates in some important cellular processes and can also intervene in the occurrence of tumors. The data from relevant studies suggest that PCNP is highly expressed in several malignant tumors, just like cervical cancer, rectal cancer, and lung cancer. Related studies have confirmed that there is a mutual relationship between PEST protein and some functional proteins such as transcription factors, cyclins, and proto-oncogene expression proteins [17]. PEST proteins are widely distributed and involved in other cellular functions, such as the ubiquitin–proteasome pathway, nuclear pore glycosylation, and hexose biosynthesis pathway, as well as in the regulation of the cell cycle and cell proliferation. These proteins are also associated with chromosomal stability, tumor occurrence, and the immune system.

In this review, this manuscript discuss the involvement of PCNP ubiquitination in two related signaling pathways as well as the influence of upregulation and downregulation of PCNP in related cancers and its mechanism of action (Fig. 2).

## Molecular Biological Mechanism of PCNP

### PCNP and Ubiquitination

Ubiquitination [18, 19] is the most active and highly controlled biological phenomenon in cells, and it degrades the labeled protein, controls its activity, changes its localization, and strengthens or hinders its interactions. The specific mechanism of ubiquitination is to first consume 1 molecule of ATP to activate ubiquitin, and then E1 ubiquitin activating enzyme binds with it to form a complex [20]. Subsequently, it was transferred to the E2 ubiquitin-binding enzyme to form a complex. When E3 ubiquitin ligase specifically recognizes substrate proteins, it catalyzes the covalent transfer of ubiquitin to target substrate proteins and undergoes proteasomal degradation. In cyclins containing the PEST sequence [21], in the late G1 phase, the E3 ubiquitin ligase ubiquitinates cyclin E and activates it [22]. Moreover, cyclin E can enhance the entry of S phase and DNA replication. The ubiquitination site in the PEST sequence appears to be lysine [23]. Proline can affect the construction process of multi-ubiquitin chain [24].

Ubiquitination can be involved in and interfere with cell survival and differentiation, cell cycle progression and many other physiological processes. Previous research has also confirmed that ubiquitination can regulate tumor growth inhibition and tumor growth promotion pathways.

NIRF is also an E3 ubiquitin ligase [4, 5]. PCNP is mutually linked with NIRF. In vitro and in vivo experiments have shown that PCNP is ubiquitinated by NIRF. Indeed, a study showed that PCNP undergoes ubiquitination in HEK-293 T cells and COS-7 cells in vivo experiments [7]. Moreover, in vitro experiments can lead to cell cycle arrest in G0/G1 phase by forcing the expression of let-7a or knocking out NIRF [25]. And the experimental results obtained from yeast two hybrid screening indicate the mutual influence between NIRF and PCNP [6]. NIRF participates in the ubiquitination of PCNP as its substrate [19]. NIRF ubiquitinates PCNP through E3 ligase. In neuroblastoma [14], both the PI3K/AKT/mTOR and MAPK pathways are regulated by ubiquitinated PCNP.

### PCNP and the Signaling Pathway

Related studies have shown that PCNP is associated with cell cycle regulation during tumor growth, as well as the PI3K/AKT/mTOR and Wnt signaling pathways. The PI3K/AKT and mTOR signaling pathways are closely connected. They are two important intracellular signaling pathways related to various aspects of cell function, participating in normal physiological activities and the occurrence of various pathological diseases [26]. In some cases, they are considered to be a unique pathway critical for cell cycle regulation. For example, regulating cell growth, proliferation, metabolism, and movement. In addition, important studies have shown that genes related to this pathway are activated in the body of cancer patients [27].

#### PCNP and the PI3K/AKT/mTOR Signaling Pathway

Serine/threonine kinase AKT, also known as protein kinase B (PKB), is the focus of thousands of studies in various fields of biology and medicine. The AKT network is almost involved in the physiological functions of every organ system.

The development of cancer is a connection with dysregulation of diverse cell signaling pathways aroused by epigenetic and certain genetic alterations. The PI3K/AKT/mTOR pathway is a main dysregulation pathway of multiple cancers [28]. This pathway includes PI3K and its downstream molecule AKT (PKB). This pathway has the function of regulating signal transduction, controlling cell proliferation, apoptosis, metabolism, and influencing angiogenesis, and it is involved in related biological processes [29]. Compared to other signal transduction pathways, it is more complex. Regulating the PI3K/AKT/mTOR signaling pathway can play a therapeutic role in ischemic brain injury, neurodegenerative

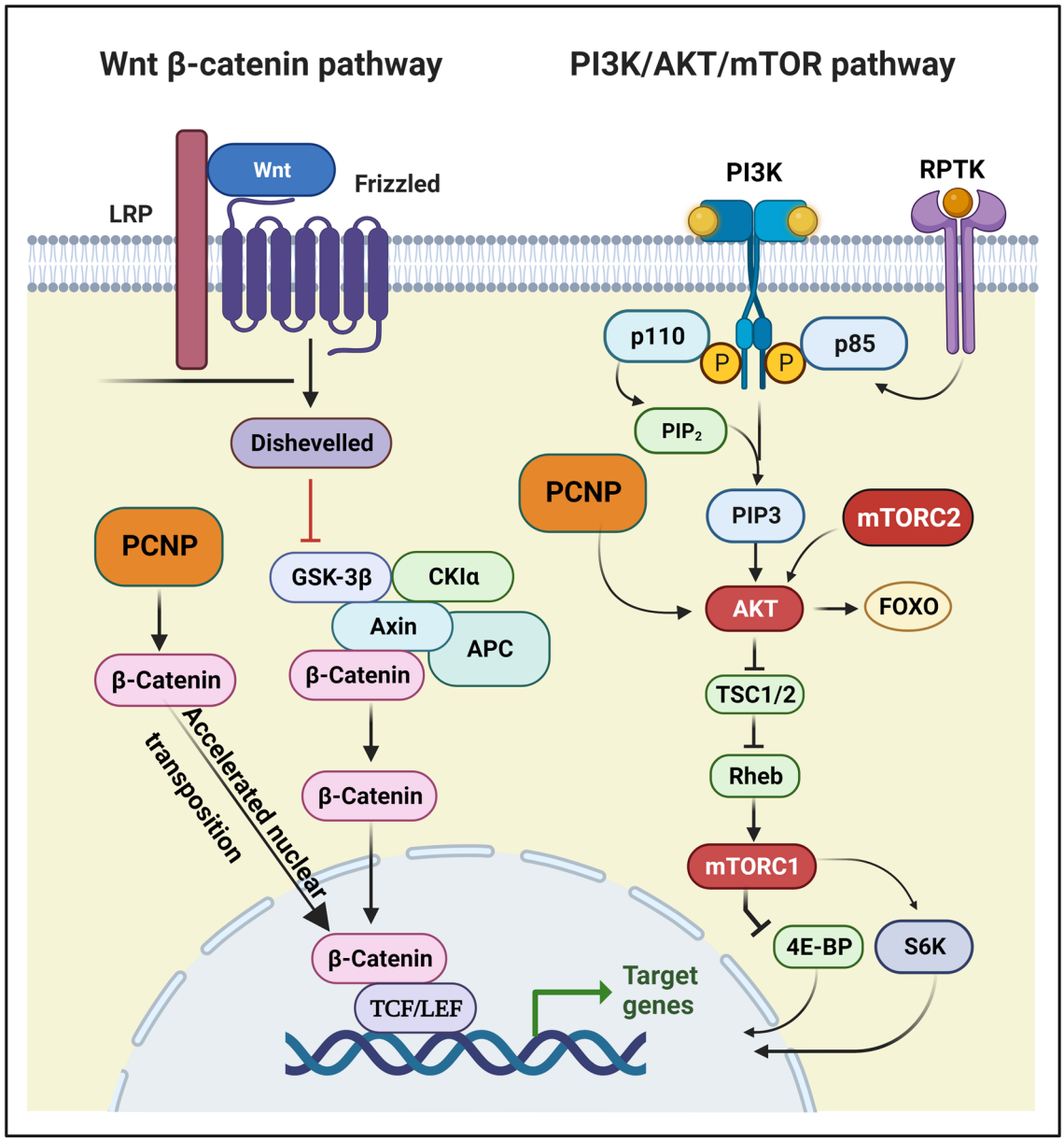


**Fig. 2** The PI3K/AKT/mTOR and Wnt signaling pathway for PCNP (Created with Biorender.com)

diseases, and tumors [30]; hence, it is considered an important pathway in various cancers.

In the pathological process of cancer, extracellular signaling molecules activate the PI3K component in the mTOR signaling pathway, causing changes in PI3/AKT/mammalian targets, achieving the goal of regulating cellular processes such as angiogenesis and metastasis, cell cycle [31]. Among the three subtypes of PI3K, type I PI3K is formed by a dimer of regulatory catalytic subunit p110 and subunit p85, where the p85 regulatory subunit can bind to tyrosine receptors on the cell membrane [30]. Tyrosine receptors bind and activate with related ligands; thus, PI3K is activated by interaction with growth factor receptors or junction proteins. This binding further promotes the p110 subunit to catalyze the conversion of PIP2 to PIP3. PIP3 can bind and phosphorylate Ser473 and Thr308 on the Akt sequence of intracellular signaling proteins, causing Akt protein activation [32]. Activating Akt can upregulate PTEN tumor suppressor genes. Upregulation of PTEN promotes PTEN nuclear translocation, which induces cellular autophagy by activating the p-JUN-SESN2-AMPK pathway [33]. Akt shows a high expression in, breast cancer, lung cancer and esophageal cancer.

The expression level of Akt can interfere with the differentiation of cancer cells and lymph node metastasis. S6 is a downstream effector of the Akt/mTOR pathway. In non-small cell lung cancer, high expression of phosphorylated S6 is associated with cancer lymph node metastasis [34]. This indirectly indicates that the expression of Akt affects lymph node metastasis. Activating Akt upregulates PTEN and promotes PTEN nuclear translocation, which can induce cell autophagy [33]. This also indirectly indicates that the expression of Akt affects the differentiation of cancer cells. Related studies have shown that PI3K and Akt can intervene through the expression of VEGF and HIF-1 during tumor growth and angiogenesis [35].

The PI3K/AKT/mTOR pathway is regulated by various upstream signaling proteins and modulates many of the downstream effectors through collaboration with several compensatory signaling pathways. Moreover, various inhibitors exist that can inhibit the PI3K/AKT signaling pathway, which can inhibit Akt expression, arrest cancer cell growth in the G0/G1 phase, and induce apoptosis [36]. In addition, relevant PI3K inhibitors [37] and drugs can also inhibit the transmission of the PI3K/AKT/mTOR signaling pathway to inhibit the proliferation of squamous cell carcinoma (OSCC) cells, such as LHPP [38], FERMT1 [39] and curcumin nanoemulsions [40]. Furthermore, Methyl lucidone (ML) can inhibit the PI3K/Akt pathway, causing G2/M phase arrest and apoptosis in ovarian cancer cells [41]. And ferruginol diterpenoids [42] and kirenol [43] can selectively inhibit the human thyroid cancer cell by mediating the PI3K/AKT signaling pathway.

PCNP also affects lung adenocarcinoma cells [16]. After upregulation of PCNP, the expression levels of p-Akt, p-mTOR and p-PI3K in lung adenocarcinoma cells can be increased to promote autophagy. In addition, upregulation of PCNP can promote tumor growth in xenograft lung adenocarcinoma, and downregulation of PCNP can regulate angiogenesis, thereby inhibiting the growth of lung adenocarcinoma cells. Upregulation of PCNP reduced the proliferation, migration, and invasion of neuroblastoma, however, downregulate PCNP reversed this effect [14]. Thus, PCNP can participate in regulation as a cell cycle regulatory protein, tumor regulatory nuclear protein, and transcription factor.

### PCNP and the Wnt Signaling Pathway

The Wnt signaling pathway is widely involved in the regulation of cell proliferation, apoptosis, and EMT, and is therefore considered an important pathway for studying tumor occurrence and development [44], with great research significance [45].

The Wnt signaling pathway is the classic pathway mediated by $\beta$-catenin. Wnts are cysteine-rich glycoproteins that regulate bone development in embryos and promote osteogenesis in adults. The Wnt signaling pathway consists of three parts. They are the nucleus, cytoplasm, and extracellular signaling components. The Wnt signaling pathway can participate in and intervene in many cancers; hence, it is considered a primary target for therapeutic interventions, for example, Wnt secretion by epithelial cells drives colon cancer progression [46, 47]. Moreover, in cancer, autocrine Wnt signaling can play a role in promoting tumor growth by increasing the proliferation and survival rate of tumor cells, for example, such as functions to promoting cell proliferation and preventing apoptosis [48, 49].

One of the important biological indicators for detecting whether Wnt is activated is β-catenin. After receiving the Wnt signal from the transmembrane receptor FZD protein family, the degrading activity of $\beta$-catenin can be inhibited by the phosphorylation of downstream protein kinases. Stable accumulation in cytoplasmic $\beta$-catenin proteins can bind to the related transcription factor and initiate transcription of downstream target genes.

Studies have confirmed that the Wnt signaling pathway plays an important part in normal growth and tumorigenesis [50, 51]. This pathway can control cell proliferation, differentiation, and apoptosis. Moreover, the Wnt signaling pathway participates in almost all stages of cancer, and the disruption of this pathway leads to several abnormalities [52]. When specific mutations occur in the components of the Wnt signaling pathway, this specific mutation can be regulated by $\beta$-catenin-mediated gene transcription disorders mediated by catenin intervene in the development and progression of many cancers, induction of tumor formation, and promotion of tumor progression. The main reason [53] for the increase in malignancy-related mortality is transition of cancer cells.

The progression of cancer metastasis is driven by multiple tumor intrinsic mechanisms; however, the role of tumor exogenous elements in the tumor microenvironment (TME) cannot be ignored, for example, macrophages in the TME usually show a high correlation with poor patient prognosis [54]. The population composition of macrophage is derived from two sources: bone marrow and tissue. Alveolar macrophages (AMs) are a population of control tissue-receptor macrophages (TRMs) involved in homeostasis and metastasis in tissues. The Wnt/β-catenin signaling pathway is a cancer cell marker and has also been identified as a pathological regulator of infection of AMs.

To gain the ability required to form metastasis, epithelial stem cells or differentiated epithelial cells must undergo an epithelial-to-mesenchymal transition (EMT) [55, 56]. Among them, $\beta$-catenin interacts with TCF/LEF and various coactivators to drive the transcription of key programs in CSC, tumor cells and EMT. For example, PCNP promotes the progression of ovarian cancer [15] by accelerating the nuclear accumulation of $\beta$-catenin and triggering cancer

cells to undergo EMT. Mechanistically, $\beta$-catenin nuclear translocation can activate Wnt/$\beta$-catenin signaling pathway, while PCNP-binding $\beta$-catenin can promote its nuclear translocation.

In addition, Wnt signal can also be regulated [48, 57] function of cell proliferation and apoptosis, thus cancer occurring is mediated by the Wnt/$\beta$-catenin target genes. The Wnt/$\beta$-catenin pathway can participate in and regulate epithelial mesenchymal transition [58], one of the major pathways of EMT, while PCNP can control the expression of genes that regulate EMT and the occurrence of EMT. CD31 is considered an ideal biomarker for endothelial cells in blood vessels. The research results indicate that through IHC experiments, the expression levels of PCNP, Ki-67, and CD31 in the PCNP knockout group were significantly reduced, indicating that PCNP downregulation inhibits endothelial growth, and vice versa. This study, it can be found that ovarian cancer cells with PCNP overexpression will change their morphology, and prove that PCNP promotes the growth of vascular endothelial cells in cancer tissue. Thus, these showed that PCNP may influence tumor through the classical Wnt signaling pathway [15]. PCNP regulates autophagy in human thyroid cancer cells by the Wnt/β-catenin signaling pathway [59]. The quantitative results of cell cycle arrest effect and cell cycle data measured by flow cytometry showed that the protein levels of autophagy markers Beclin 1 and p62 in the upregulated group of PCNP decreased, LC3A/B increased, while the opposite was observed in the downregulated group of PCNP. The specific mechanism is as follows: PCNP improves cell cycle arrest and affects apoptosis by regulating the expression of cell cycle regulatory genes and activating the ERK/JNK/p38 pathway in thyroid cancer (TC) cells. And overexpression of PCNP reduces the expression level of the Wnt/$\beta$-catenin pathway in TC cells, thereby promoting autophagy in thyroid TC cells. In addition to PCNP, hirsutenone can also inhibit Wnt/β-catenin signaling pathway induces apoptosis in human thyroid cancer cells.

### Ubiquitination of PCNP Regulates Cell Cycle

The localization of the *NIRF* gene [60, 61] at the point responsible for chromosomal DNA amplification in some types of tumor cells indicates that NIRF plays a role in cell cycle regulation and tumorigenesis in certain human tumors. NIRF is also a nuclear protein containing PEST, which ubiquitinate PCNP [17] and is involved in cell cycle regulation. Moreover, overexpression of NIRF can result an increase in cell in the G1 phase [10]. PCNP also participates in the ubiquitination process of proteins as a substrate of NIRF. Therefore, it can be inferred that PCNP is also involved in cell-cycle regulation.

### Relationship Between PCNP and Other Proteins

The selective expression of genes determines the function of the encoded proteins, however, documented evidence has demonstrated that genes encoding proteins in the same pathway or components of the same protein complex are typically co-regulated [62], and exhibited the same expression pattern. Based on the current research progress, the relationship between PCNP and the transcription patterns of related proteins is summarized below by reading the literature. On the basis of the similarity of their functions, PCNP is thought to co-express with protein phosphatase 1 (PP1CC) [63], EF hand domain protein 1 (CGREF1) [64], signal recognition particle 9-kDa protein (SRP9) [65], and phosphate polysaccharide mannose transferase subunit 1 (DPM1) [66]. According to the transcription pattern of PCNP, the results showed that PCNP was co-expressed with PSMC6, TRAM1, BMI1, MARCH7 and TMEM123 [7]. PSMC6 can remove damaged or misfolded proteins that may impair cellular function, as well as proteins that no longer require function, in order to maintain protein dynamic balance [67].TRAM1 can mediate endoplasmic reticulum membrane stimulation to secrete proteins and their transport [68]. BMI1, a proto oncogene that can form PCG-PRC1 complexes, can mediate ubiquitination of histones, leading to genetic changes in chromatin expression rate. The specific mechanism is influenced by chromatin remodeling and histone modification [69]. MARCH7 can regulate DNA damage and mediate TGF-β-induced cellular behavior [70, 71]. TMEM123 can mediate tumor cell death by inducing swelling and vacuolization of cancer cells and their organelles, as well as improving membrane permeability. The functions of these proteins above indicate that their co expression pattern with PCNP is beneficial.

## PCNP and *Cancer*

Cancer, one of the main influencing factors for global population mortality rates [72]. Among the current methods for treating cancer, chemotherapy ranks first in terms of treatment effectiveness, but there are also effects of drug resistance and side effects, [17], hence it is crucial to find new treatments for cancer.

### Colon *Cancer*

It is well known that carcinoma of colon [73], a disease has a very high incidence and prevalence worldwide, which is the third most common cancer [74]. It has a very high rate of morbidity and mortality rates [75]. However, the existing treatment methods mainly include surgery and adjuvant chemotherapy, which also bring inevitable side effects to

patients during treatment [76]. Therefore, developing new methods for treating colon cancer maybe can alleviate the current situation of inevitable side effects in treatment.

Family of signal transducers and activators of transcription (STAT) protein [77] signal transducers and activators, composed of transcription factors, is responsible for regulating various molecular and cellular processes. In cancer biology, STAT3 and STAT5 [78] have received considerable attention, they are consistently activated in a variety of cancers, through intracellular signaling to transmit signals from the membrane to the nucleus to activate gene transcription. Thus, they are linked to human cancers. Because it is known that JAK/STAT signaling pathway can be involved in cell growth and immune function. Based on the biological role of this signaling pathway and cytokines, Slattery et al. have observed many statistically significant interactions of this pathway after many experiments, one of which is that STAT3 and STAT5 are correlated with colon cancer survival [79].

As a transcription factor, PCNP serves two purposes in different tumor types through regulating the signaling pathways it controls and the expression of multiple genes. One study demonstrated that PCNP is associated with lymph node metastasis in colon cancer [80]. Thereafter, based on the recognition that PCNP is a differentially expressed gene associated with lymph node involvement in colon cancer, and that overexpression of PCNP upregulates the signal transducer and activator of transcription (STAT)3/5 pathway and inhibits apoptosis in human colon cancer cells, Xu et al. demonstrate that LINC00858 upregulates PCNP by cumulating the transcription factor RAD21, activates the STAT3/5 signaling pathway, and promotes colon cancer progression. The authors thought that LINC00858 may affect the progression of colon cancer through the STAT3/5 pathway, which is modulated by PCNP and RAD21 [81].

In addition, Wnt/$\beta$-catenin [82, 83] and PI3K/AKT/mTOR [84, 85] signaling pathways are also involved in the regulation of colon cancer [86]. Thyroid hormone receptor $\beta$1 can promote the development of human colorectal cancer by enhancing PI3K/AKT signaling [87]. And scutellarin enhances Wnt/β-catenin signaling can promote colon cancer [88].

## Ovarian *Cancer*

One of the main causes affecting the mortality rate of female cancer patients worldwide is ovarian cancer [89], and almost 140 thousand [90] patients die annually worldwide. It is the most lethal tumor in the female reproductive organs [91]. In the early stages of cancer, only a very small number of ovarian cancers can be diagnosed [92], and more than half of patients diagnosed with ovarian cancer are accompanied by tumor metastasis, leading to poor prognosis and high mortality rates. Up to now, most treatment methods are single [93], and the presence of drug resistance and repeated treatments have also led to a lower 5-year survival rate for patients. Based on the difficulty in diagnosing ovarian cancer mentioned above, it is urgent to explore a new and effective biomarker and potential molecular mechanism for ovarian cancer metastasis, providing new directions for the treatment of ovarian cancer.

Kwon et al. have shown that Wnt/$\beta$-catenin signaling and its downstream pathway, epithelial-to-mesenchymal transition, are play an important part in ovarian cancer metastasis [94]. At the same time, downregulation of PCNP can inhibit the activity of ovarian cancer cells and accelerate the apoptosis of cancer cells [15]. The specific mechanism is as follows. First, PCNP combines with $\beta$-catenin, which promotes nuclear translocation of $\beta$-catenin, and the nuclear translocation of $\beta$-catenin will further activate the Wnt/$\beta$-catenin signaling pathway. In addition, PCNP regulates the expression of EMT-related genes, which subsequently promoting the emergence of EMT. These findings suggest that PCNP may promote the progression of ovarian cancer through the activation of the Wnt/β-catenin signaling pathway and EMT. At the same time, in order to investigate the effect of PCNP on the migration and invasion of OC cells, a PCNP overexpression and knockout model was established. The results showed that PCNP overexpression promoted the migration of SK-OV-3 and A2780 cells. However, downregulation of PCNP significantly inhibited cell migration. It may become a new target for the treatment of ovarian cancer. In addition, another study suggests that SOX9/NFIA can also affect Wnt/β-catenin signaling pathway promotes metastasis of human ovarian cancer [95].

## Thyroid *Cancer*

The most common case of endocrine malignancies is thyroid cancer, and papillary thyroid cancer is one of the most well-known form, which accounts for almost 85% [96], of thyroid cancer cases [97]. And the incidence of small papillary thyroid carcinoma continued a sharp increase in recent years [98]. However, despite multiple intensified treatments [99], malignant thyroid cancer still has a poor prognosis [100], and the pathological and physiological mechanisms of TC occurrence are still unclear. Therefore, in order to solve the current dilemma and better treat thyroid cancer, it is crucial to search for new oncogenic molecules that mediate cancer. Hydrogen sulfide ($H_2S$) is currently widely recognized as the third gas signaling molecule that plays an important role in various physiological and pathological conditions. Many studies have shown that many signaling pathways, such as promote autophagy, miR-30c, PI3K/Akt/mTOR [101], and LKB1/STRAD/MO25, AMPK/mTOR signaling pathways, induced by $H_2S$ [102].

Wu et al. demonstrated that exogenous $H_2S$ regulated the development of thyroid cancer cells by the RAS/RAF/MEK/ERK and ROS/PI3K/Akt/mTOR pathways [103]. However, studies have shown that PCNP can also mediate the development of thyroid cancer cells.

As important members of the MAPK family, ERK, JNK, and p38 MAPK play important roles in inducing apoptosis and cell cycle arrest [104]. PCNP, which controls the expression of cell cycle regulatory genes and activates the ERK/JNK/p38 pathway, can enhance cell cycle arrest and induce apoptosis of thyroid cancer cells. Chen et al. showed that [59] overexpression of PCNP can inhibit the growth of human thyroid cancer cells in both xenograft and non-xenotransplantation settings; moreover, downregulation of PCNP induced an opposite trend in these cells. In addition, PCNP overexpression can reduce the expression level of the Wnt/β-catenin signaling pathway components in TC cells, that is, the regulating Wnt3a and inhibiting the activation of $\beta$-catenin and GSK-3$\beta$, thus promoting autophagy in TC cells. However, after the downregulation of PCNP, the expression level of the Wnt/$\beta$-catenin signaling pathway in TC cells will increase, namely, showing the opposite result. These findings suggest that PCNP upregulation could serve as an approach to prevent the development of human thyroid cancer.

In addition, by knocking down the vitamin D receptor, the Wnt/$\beta$-catenin signaling pathway can be mediated to promote the proliferation, apoptosis and invasion of thyroid cancer cells [105], and it can also inhibit the Wnt signaling pathway by knocking out the long non-coding RNA CCAT2/β chain protein pathway to achieve anti-tumor effect [106].

## Lung Adenocarcinoma

Among global cancer cases, patients with lung cancer have the highest mortality rate [107]. According to histological classification, lung cancer can be divided into small cell lung cancer, squamous cell lung cancer, lung adenocarcinoma, and large cell cancer; among these types, lung adenocarcinoma is the main form of cancer [108]. Like ovarian cancer, more than half of lung cancer patients have metastatic diseases and poor prognosis [109]. The 5-year survival rate of patients with advanced or metastatic lung cancer does not exceed one-fifth [110]. Based on the severe situation of metastasis and poor prognosis of lung cancer, finding a new cancer biomarker to overcome the current difficulties may break the deadlock.

A recent study has shown that PCNP can regulate the STAT3/5 and PI3K/Akt/mTOR signaling pathways in human lung adenocarcinoma to intervene in cancer progression [16]. Downregulation of PCNP will inhibit the development of lung adenocarcinoma cells, while upregulation of PCNP will have a different situation. The overexpression of PCNP increase the level of expression of p-PI3K, p-STAT3/5, p-Akt and p-mTOR in lung adenocarcinoma cells, thereby enhancing autophagy, while PCNP knockdown showed the opposite trend. In addition, upregulation of PCNP promotes the development of xenograft lung adenocarcinoma; while downregulation of PCNP inhibits angiogenesis, thereby inhibiting the development of lung adenocarcinoma.

At the same time, by establishing PCNP overexpression and knockout models, that manuscript investigated the proliferation, migration, and invasion of human lung adenocarcinoma cells mediated by PCNP. The experimental results showed that overexpression of PCNP promoted the proliferation and survival ability of A549 and H1299 cells, increased the number of colonies, and promoted the migration of A549 and H1299 cells, while knockdown of PCNP showed the opposite effect. These results indicate that PCNP can mediate the proliferation, survival, migration, and invasion of human lung adenocarcinoma cells.

Therefore, these findings suggests that PCNP downregulation could prevent the development of human lung adenocarcinoma.

## Oral Squamous Cell Carcinoma

Oral cancer, the sixth most common cancer [111]. And the incidence rate of oral malignancies is increasing [112], among which squamous cell carcinoma is the most common. Oral squamous cell carcinoma is the most common head and neck malignant tumor [113] with the highest [114] incidence rate among oral cancers. The mortality rate of oral squamous cell carcinoma patients is as high as nearly half [115].

At present, the "gold standard" for the diagnosis of tumors in clinical practice is still pathological diagnosis [116]. However, due to the fact that most oral malignancies are discovered relatively late and generally diagnosed in the late stages of cancer, the cure rate for oral cancer patients is extremely low. Therefore, early diagnosis is particularly important as it can effectively prevent cancer complications and have a good prognosis. However, due to its unique pathological location, accurate and timely early detection of oral cancer is very difficult. Therefore, it is urgent to find a new cancer biomarker to overcome the current dilemma.

Multiple studies have shown that PCNP plays different roles in different cancers, sometimes acting as an oncogenic factor and sometimes as an anticancer factor. The latest study shows that in OSCC patients, the survival rate of patients with high expression of PCNP is significantly higher than that of those with low expression or normal value of PCNP, indicating a positive correlation between PCNP expression and patient survival rate. By systematically analyzing the mechanical properties of tumor differentiation and tissue interface, Zhang Leyang et al. found that the expression level

of PCNP varies in OSCC tissues with different degrees of differentiation, and the expression level of PCNP in adjacent cancer tissues is lower than that in cancer tissues. These research results indicate that PCNP can serve as a potential biomarker for diagnosing and distinguishing OSCC stages. It was also found that measuring the mechanical properties of the cancer cell tissue interface can better grasp effective information and provide strong evidence for the diagnosis of OSCC [117].

## Summary

PCNP is a finger circular protein, is a protein ligase with ubiquitination ability; it is mainly localized in the nucleus and participants in proteolytic degradation. Moreover, data from relevant studies suggest that PCNP may participate in signaling pathways related to cell cycle regulation and genomic stability through its interaction with NIRF. Thus, PCNP is involved in cell cycle regulation. The researchers also found high expression of PCNP has been observed in various cancer cell lines, which is H-937 myeloid leukemia cells and in various cancer cells, including hepatocellular carcinoma cells, colon cancer cells, ovarian cancer cells, pancreatic cancer cells, thyroid cancer cells, oral squamous cell carcinoma cells and cells of other malignant tumors. This fact suggests that PCNP may be involved in carcinogenesis. Thus, PCNP could serve as a new therapeutic target; moreover, effective PCNP inhibitors or stimulants can be designed and developed to treat related cancers. The present review summarizes the mechanisms of interaction and relationships of PCNP with other related proteins.

It is found that PCNP is related to cell cycle regulation during tumor growth and PI3K/AKT/mTOR signaling pathway. Additionally, the upregulation or downregulation of PCNP activates or suppresses cancer-related signaling pathway, thereby enabling the regulation of cancer development.

Because of the influence of tumor heterogeneity, PCNP affects differently on different tumor types. Generally speaking, tumor heterogeneity can be divided into two categories: inter tumor heterogeneity and intra tumor heterogeneity. The former refers to the differences in genes and phenotypes between cells of different tumors, while the latter refers to the differences in genes and phenotypes between cells of the same tumor. Intratumoral heterogeneity can be divided into spatial heterogeneity (different regions of the same tumor) and temporal heterogeneity (different primary and secondary tumors). If the tumor is homogeneous, all tumor cells have the same sensitivity to the given treatment, so as long as the tumor cells die more than new ones, it can be completely cured. However, the existence of tumor heterogeneity results in different subgroups having varying degrees of sensitivity to different or the same treatment methods. Therefore, clinical treatment should focus on intratumoral heterogeneity and develop personalized and precise plans.

In colon cancer, PCNP overexpression can activate and inhibit the STAT3/5 signaling pathway Apoptosis of human colon cancer cells. Moreover, in human ovarian cancer, related studies have demonstrated that PCNP can also act through activation the Wnt/β-catenin signaling pathway to promote $\beta$-catenin nuclear displacement or regulate the expression of EMT-related genes and then trigger the occurrence of EMT, thus promoting the progression of ovarian cancer. While in human thyroid cancers, PCNP shows a high expression in human thyroid cancer; this effect decreased the proliferation, migration, and invasion of xenografted TC cells. PCNP overexpressing and activating the ERK/JNK/p38 pathway and inhibiting the Wnt/$\beta$-catenin pathway, thus regulating apoptosis and changing the expression of genes regulating cell cycle to affect cell cycle arrest and promote autophagy in TC cells. Another study revealed that the progression of human lung adenocarcinoma can be suppressed by downregulating PCNP expression to reduce the expression levels of the STAT3/5 and the PI3K/Akt/mTOR signaling pathway components. In addition, relevant PI3K inhibitors and drugs can also inhibit the transmission of the PI3K/AKT/mTOR signaling pathway to inhibit the proliferation of squamous cell carcinoma cells.

At the same time, a study found a close relationship between the expression of tumor necrosis inducing protein 8-like 2 (TIPE2) and PCNP in patients with rheumatoid arthritis (RA), and both expression levels were significantly increased. In order to investigate the differential expression of TIPE2 and PCNP in peripheral blood mononuclear cells (PBMCs) of active and inactive RA patients, an observational study was conducted to compare high disease activity RA patients with low disease activity RA patients. The research results indicate that the expression levels of TIPE2 and PCNP in PBMCs of active RA patients significantly increase. This indirectly suggests that PCNP is likely to be related to autoimmune function [118].

## Perspective

Proteins containing PEST can easily become targets for ubiquitination. Among nuclear proteins containing PEST, PCNP is a short-lived new oncogene, and its ubiquitination can serve as a new chemotherapy target. Although the research potential of PCNP is large, current studies on PCNP are not comprehensive, and other regulatory mechanisms have not been investigated. Thus, further studies on this topic are required.

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## Declarations

**Conflict of Interest** The authors declare no conflict of interest.

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