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REVIEW ARTICLE

Overview of PCTK3/CDK18: A Cyclin-Dependent Kinase Involved in Specific Functions in Post-Mitotic Cells

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Abstract: Cyclin-dependent kinases (CDKs) comprise a family of about 20 serine/threonine kinases whose catalytic activity requires a regulatory subunit known as cyclin; these enzymes play several roles in the cell cycle and transcription. PCTAIRE kinases (PCTKs) are a CDK subfamily, characterized by serine to cysteine mutation in the consensus PSTAIRE motif, involved in binding to the cyclin. One member of this class is PCTK3, which has two isoforms (a and b) and is also known as CDK18. After being activated by cyclin A2 or phosphorylation at Ser12 by PKA, PCTK3 can perform several functions. Among these functions, we may highlight the following: modulation of cargo transport in membrane traffic, p53-responsive gene, regulation of genome integrity. According to different studies, PCTK3 dysfunction is related to a wide range of diseases, such as metabolic diseases, cerebral ischemia, depression, cancer, neurological disorders, and Alzheimer's disease. Although this protein participates in different biological events, we may say that PCTK3 has received far less attention than other CDKs. There are thousands of published articles about other CDKs and less than two hundred articles related to PCTK3. The main objective of this review is to present the selected published studies about this protein. Our focus is on PCTK3 particularities compared to other CDKs. Here we give an overview of the biological functions of PCTK3 and explore its potential as a target for drug design.

Keywords: CDK18, PCTAIRE 3, DNA replication stress, transcription, cancer, Alzheimer's disease, PCTK3.

1. INTRODUCTION

Cyclin-dependent kinases (CDKs) (EC 2.7.11.22) are a family of 20 serine/threonine kinases that act directly or indirectly on the regulation of the cell cycle progression (CDKs 1-6, 11, 14-18), transcription of DNA (CDKs 7-10, 12, 13, 19, 20), and post-mitotic events (CDK5, CDK18) [1-33]. The CDK family requires a specific regulatory subunit and/or post-translational protein modification for full activation [34].

Functional studies indicated that heterodimeric cyclin-CDK complexes regulate cell cycle progression [35, 36]. All CDKs contain a highly conserved PSTAIRE motif, which is mandatory for the interaction with activating proteins, called cyclins [35]. The oscillation in the synthesis and degradation of cyclins attached to their respective CDK partners drive the transition from one cell cycle phase to the next [36].

CDKs can be named numerically or according to a highly conserved amino acid sequence in the PSTAIRE motif of cdc2, which is the first member of this

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protein kinase family [37]. Until studies revealed the association of cyclins to these proteins, they received names following the sequence of their PSTAIRE motif: PCTAIRE 1-3, PFTAIRE, PITAIRE, KKIALRE, PISSLRE, and the PITSLRE [38].

According to sequence similarities, phylogenetic analysis of CDKs reveals that we can study these enzymes in smaller groups or subfamilies. We identify one of these subfamilies by its consensus sequence PCTAIRE. This subfamily has three members, the enzymes CDK16 [39-41], CDK17, and CDK18, which are also known as PCTK1, 2, and 3, respectively [42]. The main sequence similarity in this subfamily is a Ser to Cys mutation in the family consensus motif PSTAIRE, which is relevant to cyclin binding and, consequently, to CDK activation [43].

The focus here is on PCTK3, a protein that belongs to the CDK family and has functions beyond the CDK roles as cell cycle/transcription regulators [44]. PCTK3 is involved in different biological events, especially in brain cells, which implies that several diseases may be associated with dysfunction of its regular activities. Since its discovery in 1992 [37], we may say that we have few publications about PCTK3. While other CDKs received attention in the last decades, there is still a lot to know about PCTK3. Therefore, this article aims to review the research about PCTK3. We summarize the current knowledge of PCTK3 structure, functions, and relation to diseases. We also emphasize its potential as a target in drug development.

2. PRIMARY STRUCTURE, SUBUNIT COMPOSITION & STRUCTURE OF THE PCTAIRE PROTEINS

The main feature of the CDK subfamily PCTK is the replacement of the serine residue by one of cysteine in the PSTAIRE motif, which is conserved in the first characterized CDKs. One study suggested that this replacement could prevent the binding of PCTKs to cyclins or provide a unique binding site for another co-factor [43]. Another study showed that we activate PCTKs through protein kinase A (PKA) and the binding of cyclin A2 [45].

PCTK genes are conserved in eukaryotes, from Dictyostelium slime fungi, parasitic trypanosomes, and nematode worms to fish, birds, reptiles, and mammals [37, 43, 45-48]. In upper eukaryotes, three PCTK kinases have been described, called PCTK1, PCTK2, and PCTK3 [43]. PCTK1, PCTK2 and PCTK3 human genes are located on chromosome X (Xp11.3-p11.23) on chromosome 12 (12q23.1), and chromosome 1 (1q31 - q32), respectively [43, 49].

By 2009, we had two isoforms for both PCTK1 and PCTK3, while for PCTK2, we found only one [43]. Currently, we know three PCTK1 isoforms (1, 2, and 3), two PCTK2 isoforms (1 and 2), and two PCTK3 isoforms (a and b); their sequence information is deposited into NCBI databases and available at the website (https://www.ncbi.nlm.nih.gov/). Fig. (**1**) shows size similarities and differences in the amino acid sequences of PCTKs.

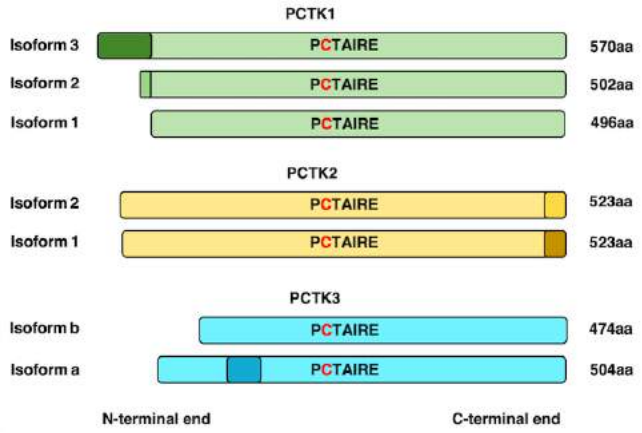

Fig. (1). Scheme of human PCTKs isoforms. PCTK1 has three isoforms: the smaller one is isoform 1, which contains 496 amino acid residues; isoforms 2 and 3 have 6 and 74 extra residues in the N-terminal end, which results in a total number of 502 and 570 residues, respectively. Both PCTK2 isoforms (1 and 2) have the same number of amino acid residues, 523 each, but they differ in the last 11 residues of the C-terminal end. PCTK3 has two isoforms (a and b) that differ in size: isoform b has 474 residues, and isoform a has 30 extra residues inside the sequence, containing 504 residues overall. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

The kinase domain of human PCTKs proteins has a relatively high sequence identity with the kinase domain of human CDK2. For PCTK 1, the identity is 53%, for PCTK 2 is 51%, and for PCTK3 is 52% [37, 43, 47]. All PCTKs contain the VALK motif in subdomain II, which directs ATP to the active site. The HRD motif in subdomain VIb shows the aspartic acid involved in phosphotransfer. The DFG motif is responsible for coordinating the Mg^{2+} ion and ATP in subdomain VII [43].

Of all human PCTKs, only PCTK1 has its crystal structure partially solved and deposited in the Protein Data Bank (PDB) [50-52]. There are two structures (PDB IDs: 5G6V and 3MTL) containing the same sequence, which corresponds to the kinase domain. This domain is conserved in its three isoforms and excludes part of the N-terminal end.

Fig. (2). PCTK3 model created from PCTK1 structure (PDB ID: 5G6V) as a template, using the program MODELLER, and alignment of PCTKs 1 and 3 kinase domain sequences. The main differences between the sequences of these two proteins are located in the C-terminal region (highlighted in a **light blue box**). Important conserved regions are the PCTAIRE helix (highlighted in the **gray box**), the VALK motif in subdomain II (**yellow box**), the HRD motif in subdomain VIb (**green box**), and the DFG motif in subdomain VII (**orange box**). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

A sequence alignment of the PCTK1 crystal structure (PDB ID: 5G6V) and PCTK3 isoforms reveals that the kinase domain is the only region in PCTK3 that we can model using the PCTK1 data as a template. Homology modeling using the program MODELLER [53-55] was able to generate a model for PCTK3 using PCTK1 as a template. Analysis of the PCTK3 sequence indicated differences in the C-terminal end. On the other hand, the region near the ATP binding site shows good agreement (Fig. **2**) between sequences of the model and template.

One characteristic that differentiates PCTKs from the other CDKs is the Ser to Cys mutation in the CDKs consensus motif PSTAIRE, as depicted in Fig. (**3**), in which PCTK3 isoforms and CDK2 are compared. From Fig. (**3**), it can also be observed that CDK2 is essentially composed of the kinase domain, while PCTKs present N- and C-terminal domains in addition to the kinase domain, being the N-terminal longer than the C-terminal one [56, 57].

During evolution, PCTKs in different organisms diverged in size. The first appearance of an N-terminal domain on these proteins occurs in organisms of the phylum Platyhelminthes. The conservation of this domain in all higher organisms and its absence in PCTKs of lower organisms correlates with the phylum Platyhelminthes being the first to have a centralized and complex nervous system [43]. This conservation in higher organisms suggests a role in the regulation and coordination of the central nervous system.

PCTKs expression pattern varies in an organism: PCTK1 is widely expressed in mammalian tissues, while PCTK3 is expressed in many but not all tissues. We find PCTK2 mostly in the brain and lung. They are relatively more abundant in post-mitotic cells. This result suggests that these proteins may also have functions that are not related to the cell cycle [43, 45]. Also, post-mitotic neurons express these proteins. These observations indicate that they have specific functions in this type of cell. However, the biological functions of PCTKs in the brain are not elucidated yet [43].

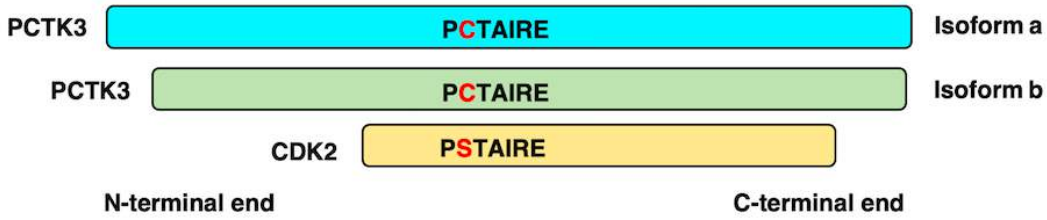

Fig. (3). Comparison of size among PCTK3 isoforms and CDK2. The Ser to Cys mutation that characterizes the PCTK subfamily is highlighted in red. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*)

3. PCTK3 PCTAIRE INHIBITORS

The well-recognized role of CDKs in cancer pathology makes them attractive targets for inhibitors [58]. We can classify CDK inhibitors as broad-range inhibitors (such as Flavopiridol, Olomoucine, Roscovitine, Kenpaullone, SNS-032, AT7519, AG-024322, R547), specific inhibitors (such as Fascaplysin, Ryuvidine, Purvalanol A, NU2058, BML-259, SU 9516, Palbociclib, Riviciclib hydrochloride), and third-generation inhibitors (such as CR8#13 and Dinaciclib) (Fig. **4**) [59].

ATP-competitive compounds constitute the most studied CDK inhibitor class, being by far the most numerous. These molecules can bind partially or totally to the ATP-binding pocket [4, 58]; however, most of them are not highly selective (an example is Roscovitine, which inhibits CDKs 1, 2, 5, and 7 successfully, but is a weak inhibitor for CDKs 4 and 6) [59].

Among CDK inhibitors, those focused on inhibition of CDK4 and 6 showed anticancer efficiency. These drugs used with aromatase inhibitor Letrozole can treat estrogen-receptor-positive breast cancer [60]. Palbociclib was the first CDK inhibitor approved by the US Food and Drug Administration (FDA) in February 2015; in the following years, the agency also approved

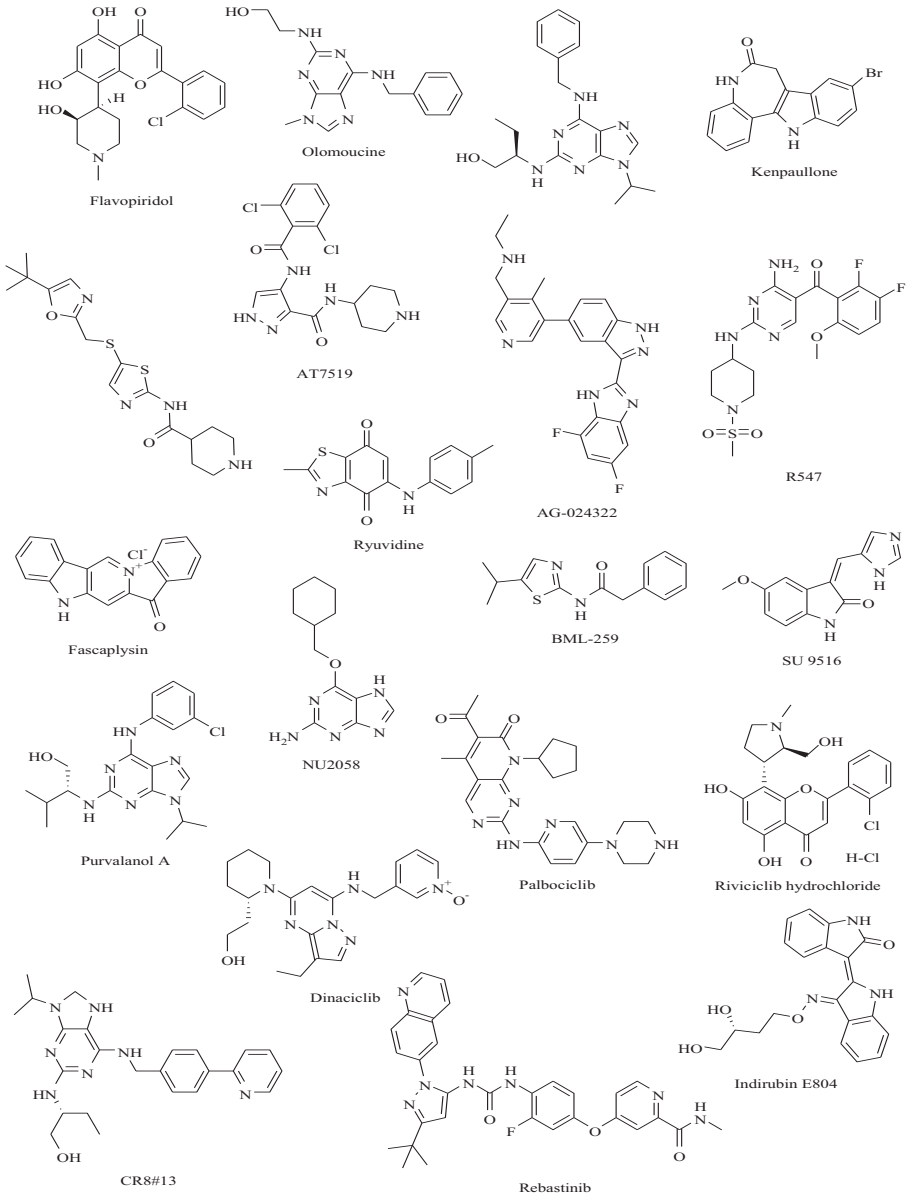

Fig. (4). Chemical structures of identified CDK inhibitors.

the compounds Ribociclib and Abemaciclib. These approvals are significant achievements, but the search for other specific inhibitors, especially for the other CDKs, needs to continue [58].

Studies about PCTKs inhibitors are recent and lack information. PCTK1 (CDK16) is the only PCTAIRE with an available crystal structure, and it is the most studied within the subfamily. Dixon-Clarke *et al.* [41] found out that, among the compounds tested for PCTK1 inhibition, the most potent were Dabrafenib and Rebastinib. They also observed that the conformational plasticity found in the PCTK subfamily allows the protein binding to type I and type II non-specific kinase inhibitors (Indirubin E804 and Rebastinib, respectively) [41], as shown in Fig. (**5A** and **5B)**.

All CDKs have a conserved activation loop (represented in blue in Fig. (**5A**) and green in Fig. (**5B**)) that contains the motif DFG (Asp-Phe-Gly). This region is a common regulatory motif in kinases and is also known as the magnesium positioning loop because the DFG aspartate coordinates one of the two Mg^{2+} ions [61]. These ions are essential for ATP binding in the active site.

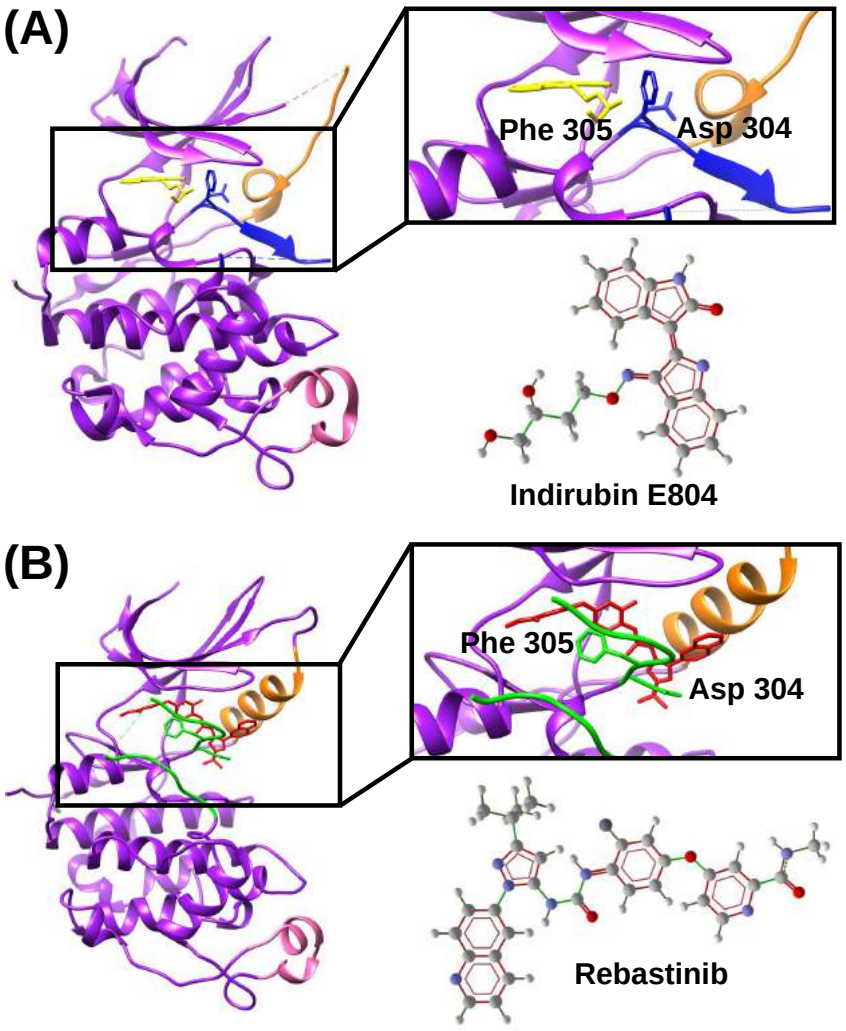

Fig. (5). (A). Structure of the PCTK1 kinase domain solved in complex with Indirubin E804 (PDB code: 3MTL). **(B)** Structure of the PCTK1 kinase domain solved in complex with Rebastinib (PDB code: 5G6V). In gray, PCTK1 crystal structure; in yellow, Indirubin E804 (type I inhibitor) molecular structure; in red, Rebastinib (type II inhibitor) molecular structure; in orange, PCTK1 αC helix; in pink, CDK/MAPK insertion; in blue: DFG-in binding conformation; in green: DFG-out binding conformation. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

When the protein is in its active conformation, the phenylalanine residue in the DFG motif (Phe305 in Fig. (**5**)) is between residues from the C and N-lobes; these residues' orientations create a hydrophobic pocket that is called "DFG-in" conformation (Fig. **5A**). However, when this Phe residue moves its side chain out of the hydrophobic pocket, the DFG aspartate (Asp304 in Fig. (**5**)) also re-orientates, leading to a disruption in the Mg^{2+} coordination, and in some cases to a steric blockage of the ATP binding site; in these conditions, the protein kinase is inactive and in a "DFG-out" conformation (Fig. **5B**) [61]. Type I kinase inhibitors compete directly for the ATP binding site and bind to the "DFG-in" conformation (represented in blue in Fig. **5A**), in which PCTK1 is in its active form. Type II inhibitors bind to the "DFG-out" conformation (represented in green in Fig. **5B**), in which the protein is in its inactive form [41].

The plasticity found in PCTK1 was associated with several factors, such as the interaction between the PCTKs and its corresponding cyclins facilitated by a 'PCTAIRE' sequence motif in the kinase αC helix that is conserved in this subfamily but diverges from the classical 'PSTAIRE' motif found in CDK2. Besides it, PCTKs present a partially inverted DFG motif and a CDK/MAPK insert in its C-lobe (represented in pink, in Fig. (**5**)) [41]. These structural features and the chemical scaffolds provide information for the development of more selective PCTK inhibitors.

Ning *et al.* [62] reported that PARP inhibitors (PARPi) could treat glioblastoma (GBM). In this treatment method, Myc amplification in patient-derived glioblastoma stem-like cells (GSCs) leads to transcriptional repression of PTCK3, which is responsible for promoting homologous recombination (HR) and PARPi resistance by facilitating ATR activation in its DNA Damage Response signaling pathway. These researchers verified that PCTK3 knockdown suppressed HR and conferred PARPi sensitivity to GSCs, showing that PCTK3 is a promising therapeutic target for drug design [62].

Li *et al.* observed that overexpression of the CXXC finger protein 4 (CXXC4) suppresses gastric cancer cell proliferation and promotes T cell activation by negatively regulating PCTK3 in the CXXC4/ELK1/MIR100HG pathway [63]. The results reported by these two research teams show that PCTKs repression, mediated by other protein overexpression, is a promising approach for the treatment and an option for the use of PCTKs selective inhibitors.

4. CATALYTIC ACTIVATION OF PCTK3

Studying PCTK3, Matsuda *et al.* [45] identified the interaction of this protein with cyclins E1 and A2. *In vitro* assays tested the kinase activity using protein retinoblastoma as a substrate; the results showed that PCTK3 was activated only by cyclin A2 and not by cyclin E1 and that cytoplasmatic PCTK3 was able to regulate cyclin A2 stability. Analyzing the primary sequence, it was verified that PCTK3 contained four putative PKA phosphorylation sites, of which Ser12, Ser66, and Ser109 were phosphorylated by PKA in experiments *in vivo* and *in vitro*. Phosphorylation at Ser12 by PKA significantly increased the activity of PCTK3 even in the absence of cyclin A2, and in the presence of the cyclin, its activity was comparable to CDK2 [45]. Fig. (**6**) shows the two processes by which PCTK3 can be activated.

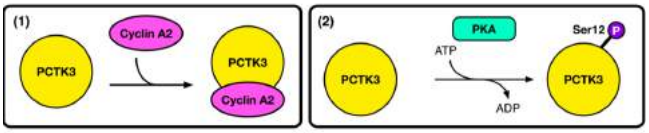

Fig. (6). Scheme of PCTK3 activation by cyclin A2 binding (1) and by PKA phosphorylation at Ser12 (2).

5. PCTK3 EXPRESSION

Analyzing the tissue distribution of the two main transcripts, PCTK3 isoforms a and b, in 12 different brain regions (frontal lobe, temporal lobe, cerebellum, hippocampus, substantia nigra, caudate nucleus, amygdala, thalamus, hypothalamus, pons, medulla, and spinal cord), PCTK3 isoform a was expressed in all regions analyzed, while PCTK3b was found in some regions: substantia nigra, thalamus, spinal cord, caudate nucleus, frontal lobe, and cerebellum. Furthermore, PCTK3a expression was always dominant, when both were expressed [47].

Although PCTKs 1, 2, and 3 transcripts are detectable in all areas of the brain, PCTKs 1 and 2 are more expressed in the olfactory bulb and hippocampus [64] while PCTK3 is predominant in the spinal cord, substantia nigra, and thalamus [47]. Besides being present in the brain, the highest PCTK3 mRNA expression was detected in the heart. PCTK3 protein was also detectable in the testis [64]. Another study determined the CDKs expression profile in the human retina and found PCTK3 maximum expression in the inner nuclear layer [65].

Regarding the heterologous expression of PCTK3, Palmer *et al.* [66] reported that they were unable to produce sufficient quantities of PCTK3 for their experi-

ments because the protein was extremely susceptible to proteolysis and was rapidly degraded after expression in *Escherichia coli*. Herskovits and Davies were successful at expressing PCTK3 by transient transfection in Chinese hamster ovary cells; however, after PCTK3 immunoprecipitation and activity *assays in vitro*, they observed that the protein is obtained in an inactive form, even using different conditions of expression/-cell host, lysis and substrates [48].

6. PCTK3 ACTIVITIES & FUNCTIONS

6.1. Membrane Traffic

Studying the COPII complex, necessary for the export of secretory cargo from the endoplasmic reticulum, Palmer *et al.* [67] verified that PCTK3 interacts directly with COPII and modulates secretory cargo transport. The recruitment of COPII to the membrane requires ATP, which can be blocked by a protein kinase inhibitor. Using two-hybrid screening, immunoprecipitation, and direct binding, the researchers observed that PCTK1 and PCTK3 interact with the Sec23p subunit of COPII. Sec23p and PCTK3 were co-immunoprecipitated from mammalian cell lysates, and it was verified that the central region of PCTK3 is the one involved in their interaction. The researchers also demonstrated that inhibition of PCTK3 activity, using a kinase-dead mutant, or specific depletion of this protein by RNAi, dramatically impacts the function and organization of the early secretory pathway [67].

6.2. p53 Responsive Gene

p53 is a nuclear transcription factor that regulates the expression of genes involved in growth inhibition, DNA repair, and apoptosis [68]. p53 gene is a classical tumor suppressor because more than 50% of human cancers have mutated p53 with loss of function [69].

Naumann *et al.* [68] identified PCTK3 as a novel p53 responsive gene. Recombinant adenoviral genomes encoding CTS-1 (a p53-derived synthetic tumor suppressor), PCTK3, and other proteins were transfected into a p53 mutant glioma cell line, and the expression of the transgenes induced by CTS-1 was confirmed by RT-PCR or immunoblot. Through flow cytometric analysis and viability assays, the researchers verified that the expression of PCTK3 mediated by CTS-1 induces glioma cell death and strongly inhibits cell growth [68].

6.3. Tau Phosphorylation

Herskovitz and Davies observed the increased PCTK3 expression in post-mortem brain tissue of human patients with Alzheimer's disease (AD) and that co-transfection of PCTK3 and Tau in CHO cells led to Tau hyperphosphorylation [48]. The study showed that PCTK3 stimulates phosphorylation of Tau at Thr231 and Ser235, and these residues are modified early in the AD pathogenesis process. The Tau protein is known to stabilize the abundant microtubules in neurons and when defective, can lead to dementia states like AD. Another important histological characteristic of AD is the accumulation of paired helical filaments, in which the abnormally phosphorylated Tau protein is observed. The study of paired helical filaments isolated from AD brain tissue detected a concentrated amount of PCTK3, suggesting an essential role of this protein in AD [48].

Also, previous studies have identified PCTK3 as a binding partner of 14-3-3 proteins [70]. This family of proteins has been localized in neurofibrillary tangles in the AD brain [71], reinforcing the importance of studying PCTK3 in AD.

6.4. Regulator of Genome Stability

The DNA Damage Response (DDR) factor ATR (Ataxia Telangiectasia and Rad3-related kinase) regulates cellular responses to replication stress to control the intra-S-phase checkpoint, lesion repair, and latent origin firing. ATR is activated in response to a variety of DNA lesions that lead to the formation of single-strand (ss) DNA, and its activity requires forming a heterodimer with its partner ATR-interacting protein (ATRIP). The heterodimer ATR-ATRIP depends on nucleofilaments formed between the replication protein A heterodimer (RPA) and ssDNA for DNA binding. Upon ATR-ATRIP binding to ssDNA-RPA, the DNA-damage-specific 9-1-1 complex (Rad9-Rad1-HUS1 clamp) binds at junctions between ssDNA and double-strand (ds) DNA with the RPA-facilitated aid of the clamp loader complex Rad17-RFC. The 9-1-1 subunit Rad9 is phosphorylated on Ser387, enabling TOPBP1 (DNA topoisomerase 2-binding protein 1) association with ATR and ATR full activation. Then, Chk1 is phosphorylated by ATR at Ser317 and Ser345, enabling a series of events: later origin firing inhibition, replication slowdown and fork stabilization, intra S-phase arrest, and G2/M arrest [72].

In 2016, studying the mechanisms that cause genome instability, Barone *et al.* [73] identified PCTK3 as a regulator of genome integrity. They verified the interaction of PCTK3 with Rad9, Rad17, and TOPBP1 in the replication stress signaling mediated by the DDR factor ATR. PCTK3 promotes phosphorylation of Rad9, but the scientists could not determine if

PCTK3 does that directly or indirectly. Cells with PCTK3 deficiency present a reduction in chromatin-bound Rad17 and Rad9 in response to replication stress; consequently, chromosomal abnormalities and an increase in endogenous DNA damage are observed. In this study, it was also found that cells depleted of PCTK3 exhibit delayed replication fork kinetics and accumulate at the beginning of the S-phase [73]. Fig. (**7**) shows a model of the replication stress signaling pathway mediated by ATR.

6.5. Control of the Actin Cytoskeleton Dynamics

Another important role of PCTK3 is in controlling actin cytoskeleton dynamics [74]. The actin cytoskeleton is important for several cellular events, such as cytokinesis, cell migration, and adhesion, and its extension and retraction are regulated by several proteins [74].

Matsuda *et al.* [45] showed that PCTK3 knockdown in HEK293T cells promoted the phosphorylation of cofilin (a protein that acts in actin filament depolymerization) and led to the accumulation of polymerized actin in peripheral areas. They also verified that the overexpression of PCTK3 mutant suppressed cofilin phosphorylation [45].

In 2017, Matsuda *et al.* [74] reported that PCTK3 knockdown increased the phosphorylation of cofilin at Ser312 and led to increased cell motility and kinase activity associated with Rho, a member of the Rho GTPase family that regulates actin dynamics.

Furthermore, they determined that PCTK3 regulates cell migration, controlling the dynamics between RhoA and Rac1 activities. It was also found that FAK (focal adhesion kinase) phosphorylation was suppressed by PCTK3, showing that PCTK3 reduces cell adhesion and migration *via* inactivation of the RhoA/ROCK pathway. PCTK3 also suppressed the interaction between FAK and focal adhesion proteins such as paxillin, integrins, and vinculin [74].

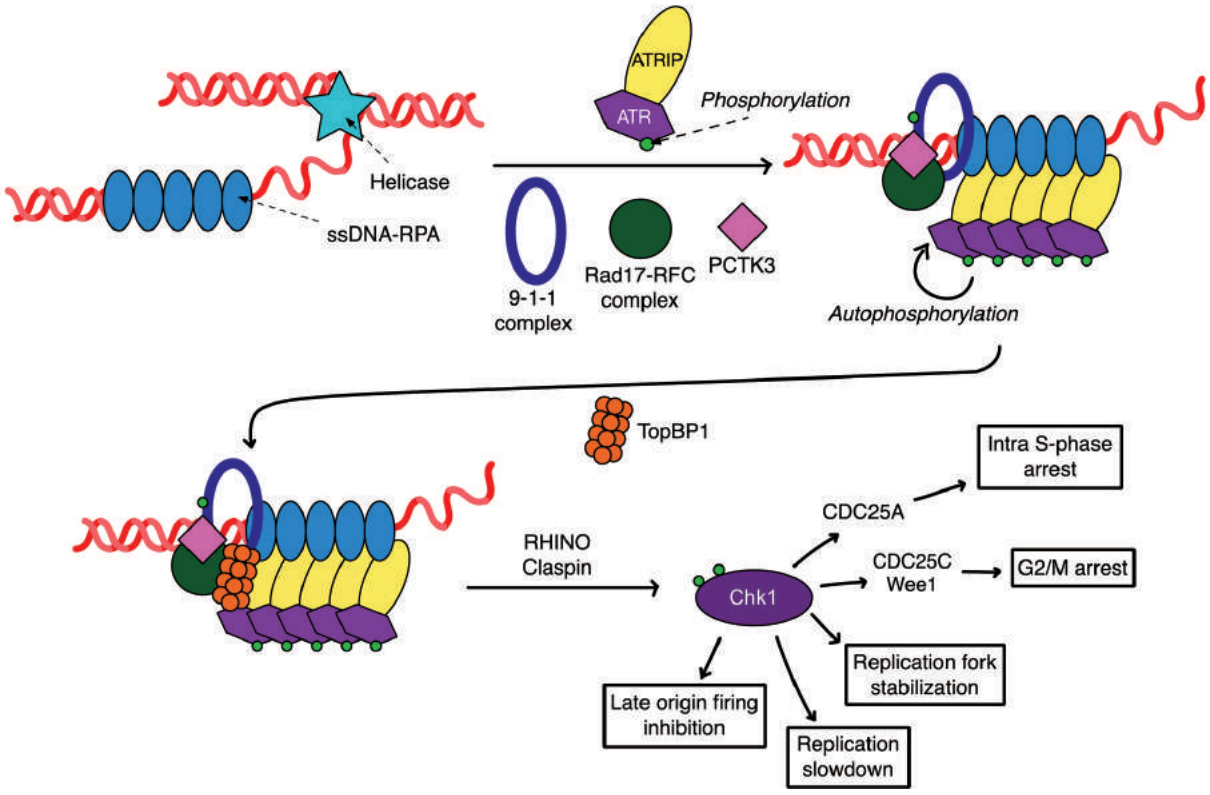

Fig. (7). Model of the ATR signaling cascade. The RPA recognizes ssDNA, formed as a result of a DNA lesion, and recruits the heterodimer ATR-ATRIP. With the aid of the Rad17-RFC complex, the 9-1-1 complex binds at junctions between ssDNA and dsDNA. PCTK3 interacts with Rad17 and Rad9 (a unit of the 9-1-1 complex) and promotes optimal Rad9 phosphorylation. Rad9 activation leads to TopBP1 binding and ATR full activation. Then, Chk1 is activated in a reaction stimulated by claspin binding to Chk1, and by the 9-1-1 complex, the protein RHINO (Rad9, Rad1, Hus1 interacting nuclear orphan), and other factors, and leads to a series of events that slow origin firing, induce cell cycle arrest, as well as stabilize and restart stalled replication forks. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

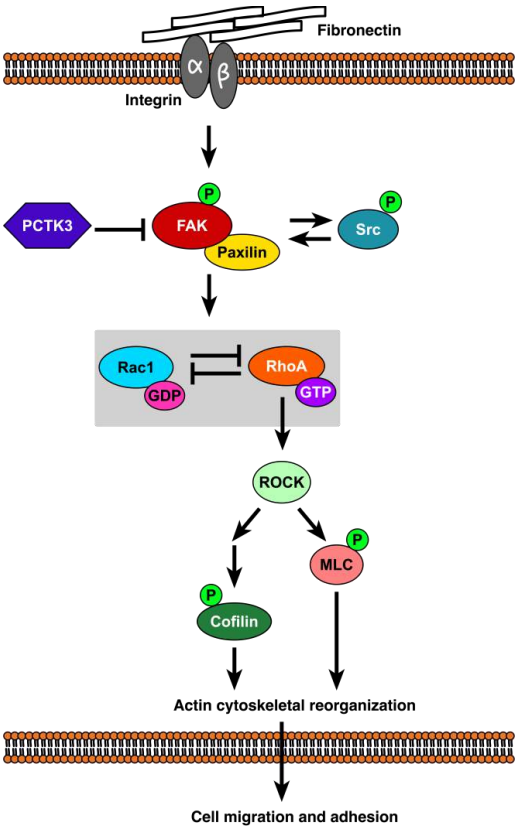

Fig. (8). Model of the regulation of FAK/RhoA/ROCK signaling pathway by PCTK3. Clusters of integrin-actin cross-linking promote FAK activation and phosphorylation at Tyr397, resulting in binding of cellular Src through its SH2-domain. FAK also interacts with adhesion proteins, such as paxillin, forming focal adhesion complexes, and regulates actin polymerization by controlling the balance of Rho GTPases (such as Rac1 and RhoA). GTP-bound RhoA activates the protein ROCK (Rho-associated kinase), which leads to MLC and cofilin phosphorylation, and consequently, to the reorganization of the actin cytoskeleton. PCTK3 regulates this signaling pathway *via* regulation of FAK activity: it suppresses the attachment-induced phosphorylation of FAK at Tyr397 and the interaction between FAK and focal adhesion proteins, leading to RhoA/ROCK inactivation and consequently reducing cell migration and adhesion. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

PCTK3 knockdown increased the MLC (Myosin-Light-Chain Kinase) phosphorylation at Thr18 and Ser19, which enhanced cell motility due to the promotion of actomyosin contractility in cells. In studies with HeLa cells, the overexpression of PCTK3 resulted in filopodia formation in the early stages of cell adhesion. In summary, the regulatory role of PCTK3 in cell motility was identified. Mechanisms of cell motility are associated with various physiological phenomena, including metastasis, and their complete understanding can help to overcome the malignant progression of tumors (Fig. **8**) [74].

6.6. Interaction with Phospholipase Cβ 1 (PLCβ1)

Scarlata *et al.* [75] found that PCTK3 binds to PLCβ1 when cells are arrested in the G2/M phase. In this study, it was discovered that, in addition to the classic function of relaying extracellular sensory information to generate intracellular calcium signals, phospholipase Cβ 1 (PLCβ) regulates PC12 cell differentiation. Analyzing this mechanism, the researchers verified that PLCβ binds mainly PCTK1, but another population of PLCβ complexes interacted with PCTK3 and cyclin B1 [75].

6.7. Differentiation of Oligodendrocyte Precursor Cells (OPC)

Pan *et al.* [76] analyzed RNA sequencing data from different neural cells. They found out that PCTK3 is highly expressed in oligodendrocytes, cells responsible for forming and maintaining myelin sheaths in axons. While other CDKs have no cell-type specificity, it was verified that PCTK3 is mainly expressed in differentiated oligodendrocytes, and the expression of this protein is upregulated during myelination and remyelination in a lysophosphatidylcholine induced model. *In vitro* assays showed that PCTK3 promotes oligodendrocyte precursor cells (OPC) differentiation by regulating the RAS/MEK/ERK signaling pathway, without significantly affecting cell proliferation and apoptosis. Overexpression of PCTK3 greatly increases ERK1/2 activation, whereas its depletion causes the opposite effect. The effect of PCTK3 overexpression on ERK1/2 is blocked when MEK1/2 is inhibited. However, the upstream effectors of PCTK3 and this protein mechanism of action are still unclear (Fig. **9**) [76].

6.8. Regulation of the Aquaporin-2 (AQP2) Water Channel

PCTK3 has been reported to control the trafficking of Aquaporin-2 (AQP2). The tight control of the abundance and localization of AQP2 contributes to the tuning of body water homeostasis. The AQP2 water channel is regulated by the hormone arginine-vasopressin (AVP), which facilitates water reabsorption in cells. When AVP binds to vasopressin V2 receptors (V2R), cAMP synthesis is stimulated and activates protein kinase A (PKA) to signal the accumulation of AQP2 in the plasma membrane of the cells and facilitate water reabsorption from primary urine. PCTK3 is required

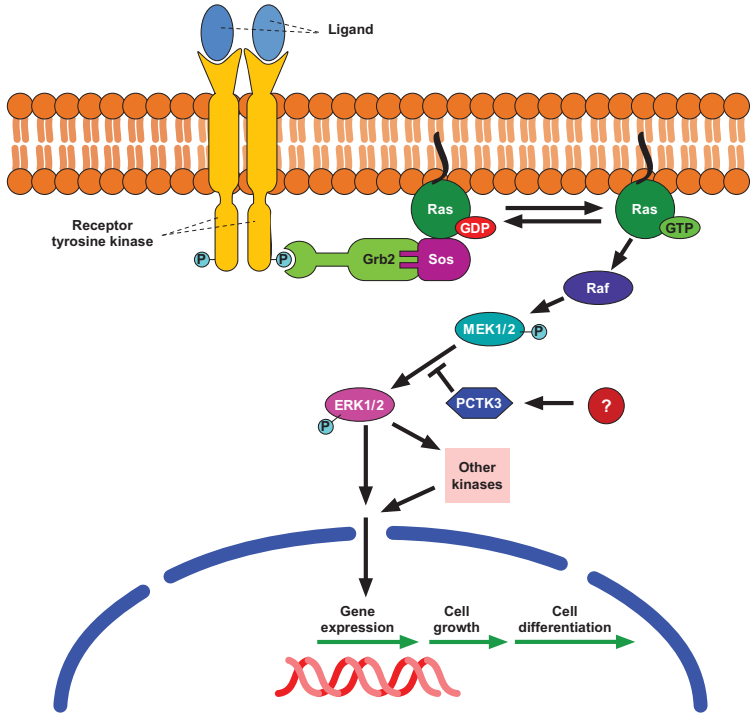

Fig. (9). Regulation of the Ras signaling cascade by PCTK3 in oligodendrocyte precursor cells (OPC). A growth factor binding to a receptor tyrosine kinase (RTK) induces RTK's cytosolic domain's autophosphorylation. The protein Grb2 binds to the RTK's phosphor-Tyr-containing peptide segment and simultaneously binds to the protein Sos. Then, Sos exchanges GDP for GTP in the protein Ras, activating this protein to bind Raf and initiate a kinase cascade. Raf phosphorylates MEK, which in turn phosphorylates ERK, which then can phosphorylate other kinases and transcription factors in the nucleus. Protein phosphatases are responsible for recycling these kinases for another cascade cycle. Therefore, through the regulation of this signaling cascade, gene expression, cell growth, and cell differentiation are modulated [78]. In OCP, PCTK3 positively influences ERK1/2 phosphorylation and cell differentiation; when PCTK3 is overexpressed, the phosphorylation levels of ERK1/2 are significantly increased, whereas PCTK3 depletion promotes the opposite effect. The upstream effectors of PCTK3 and its mechanism of action are still unclear [70]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

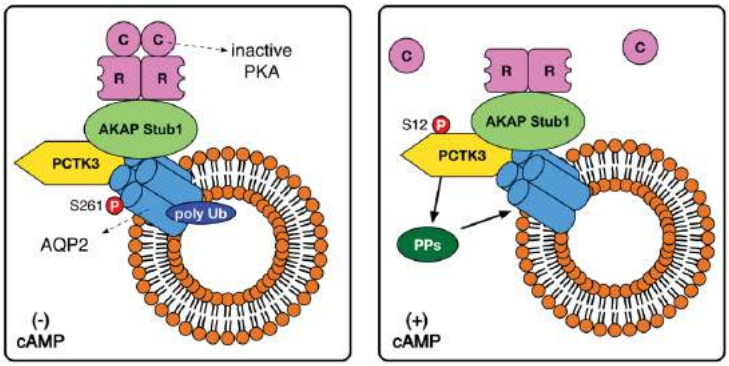

Fig. (10). Model of the regulation of AQP2 by PCTK3, PKA, and STUB1 (E3 ubiquitin ligase and AKAP). When cAMP levels are low in resting renal principal cells, PKA remains inactive, and AQP2 is retained in cytoplasmatic storage vesicles and maintained polyubiquitinated and phosphorylated (at Ser261) by STUB1 and PCTK3, respectively. After an increase in cAMP concentration, PKA is activated and phosphorylates PCTK3, which activates protein phosphatases (PPs) that dephosphorylate AQP2. The decrease of AQP2 phosphorylation and polyubiquitination facilitates the trafficking of AQP2-bearing vesicles to the plasma membrane; once incorporated into the membrane, AQP2 promotes water reabsorption from primary urine and adjusts body water homeostasis. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

for the cAMP-induced redistribution of AQP2 (Fig. **10**): it phosphorylates AQP2 at Ser261 and this phosphorylation is associated with ubiquitination of AQP2. PCTK3 knockdown reduces phosphorylation, and as a consequence, there is a decrease in AQP2 ubiquitination. The control of AQP2 abundance occurs through ubiquitin ligase STUB1, which functions as an A-kinase anchoring protein (AKAP) binding PKA to the protein complex and joining PCTK3 and AQP2 [77].

6.9. Other Important Functions of PCTK3

In 2014, screening several endogenous targets led Sahin *et al.* [79] to identify PCTK3 as a viable target to inhibit cell growth using siRNA.

Öhlinger *et al.* [80] cited the role of PCTK3 in cell cycle control of chromosomal replication and its potential in salvage pathways of pyrimidine ribonucleotides.

Also in 2019, PCTK3 was identified as an APP modifier in transgenic Drosophila and its human homologs. The initial characteristic of Alzheimer's disease is the accumulation of amyloid β (Aβ) and destabilizing the Amyloid β-precursor protein (APP) is one of the therapeutic strategies for this disease [81].

Wang *et al.* [82], researching molecular events associated with chronic cerebral hypoperfusion, studied the global expression of proteins in the hippocampus of rats and identified PCTK3 in the physiological process of chronic cerebral ischemia.

7. DISEASES RELATED TO PCTK3

As previously seen, PCTK3 is not a major driver of the cell cycle; however, it adjusts to critical cell cycle moments to ensure proper control at the checkpoint and prevent mistakes that can occur during DNA replication [36].

Recent studies [36] have detected PCTK3 as a novel important regulator of genome stability. PCTK3 depletion increases endogenous DNA damage and chromosomal abnormalities as a result of replication stress [73]. The deregulation of PCTK3 activity may support the development of several diseases including cancer and Alzheimer's Disease [83, 84].

The main diseases related to PCTK3 deregulation are summarized in Fig. (**11**).

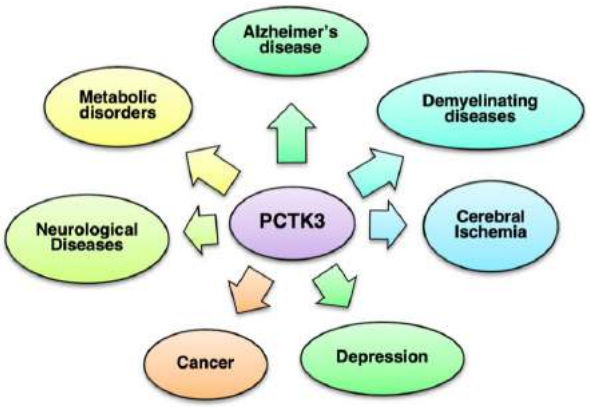

Fig. (11). Scheme of the main diseases related to PCTK3 deregulation.

7.1. Cancer

The high prevalence of CDK dysfunction and/or overexpression in human cancers is related to the control of cell proliferation [34]. Due to the cellular functions of PCTK3, exemplified by the tight regulation of ATR-mediated signaling in response to replication stress [73], this protein became a new anti-cancer drug target.

The abnormal proliferation of diseases such as cancer is the result of a compromised cell cycle, in addition to pro-mutagenic lesions [73]. For correct genome replication and precise transmission to subsequent cells occur, the ATR-mediated signaling cascade must be tightly regulated [73].

The activation of oncogenes is the result of high replication stress, exemplified in a large proportion of human cancers [85]. Through the signaling of ATR-mediated replication stress, the presence of PCTK3 is essential to avoid genome instability and the accumulation of DNA damage. PCTK3 is responsible for the retention of RAD17 and RAD9 in the chromatin, a process that is one of the main regulators of replication stress signaling [86].

Previous studies have reported the potential role of PCTK3 in the biology of cancer. Overexpression of PCTK3 was detected in about 20% of invasive breast carcinomas, 12% of metastatic prostate cancers, and 5% of serous ovarian cancer (cBioPortal), which demonstrates PCTK3 function as a cancer inducer [83, 87].

Other studies demonstrate the importance of PCTK3 in glioblastoma (GBM), a type of adult primary brain cancer, characterized by high malignancy and lethality. In GBM stem-like cells (GSCs), PCTK3 allows ATR activation and homologous recombination

(HR), making cells refractory to PARP (poly(ADP-ribose) polymerase) inhibitors. PCTK3 knockdown in GSCs results in suppressed HR and makes these cells sensitive to PARP inhibitors [62].

7.2. Metabolic Disorders

Regardless of the type of diabetes mellitus, this disease is characterized by a decline in B cell mass [88]. Patients with type 1 diabetes mellitus have an almost total loss of pancreatic beta cells, while patients with type 2 diabetes have a partial loss [89, 90].

Several studies suggest that the self-replication process maintains the pancreatic B cell mass in adult mammals [91-93]. The factors related to the control and regulation of B cell mass are not clearly understood yet; however, it is known that most cell cycle proteins are usually more associated with maintaining the number of adult B cells [88].

Taneera *et al.* investigated the CDKs expression patterns in human pancreatic islets, with and without type 2 diabetes (T2D). High expression of PCTK3 in diabetic islets was demonstrated by microarray and qRT-PCR. These results suggest that the differential expression of CDKs genes among islets from diabetic and non-diabetic donors is related to B cell proliferation, similarly in humans and other animals [88]. The information obtained in these studies may be useful to the development of an innovative treatment for diabetes that focuses on the proliferation of human B cells.

Dema *et al.* [77] performed a siRNA screening for genes related to 719 kinases and analyzed the effect of knockdown on water channel aquaporin-2 (AQP2) by high-content imaging and biochemical approaches. The result of this screening showed 13 genes highly capable of this function, including PCTK3. Through phosphorylation at Ser261 and ubiquitination mediated by STUB1, PCTK3 can control AQP2. Many diseases, such as liver cirrhosis and diabetes insipidus, which are associated with unregulated AQP2, can be favored by controlling this protein complex [77].

7.3. Neurological Diseases

In the central nervous system, CDKs act in certain processes. Both CDK5 and CDK14 interfere in the PI3K/AKT/mTOR signaling pathway but have opposite roles in differentiating oligodendrocyte precursor cells (OPC): while CDK5 promotes this process, CDK14 inhibits it [94, 95]. In addition to acting in these processes, these proteins are highly expressed in several neural cells [95]. PCTK3 expression was identified in myelinated oligodendrocytes, which strongly correlates with OPC differentiation [76].

7.3.1. Depression

A brief discussion on the role of PCTK3 related to depression was addressed in the study by Khawaja *et al.* [96]. The researchers performed proteomic analyses of hippocampal proteins and found that PCTK3 is upregulated in rats administered with venlafaxine and fluoxetine, two drugs frequently used in the treatment of depression in humans. The upregulation of PCTK3 contributed to the long-term maturation, extension of neuritis, and integration of developing granular cells within the existing hippocampal circuitry [96, 97].

Genome-wide association studies (GWAS) have revealed that major psychiatric disorders share many common alleles with small additive effects on risk [95]. Akula *et al.* [98] performed deep RNA sequencing of the human postmortem subgenual anterior cingulate cortex, a region linked to mental illness in the limbic circuits. Gene expression analyses of samples from 200 individuals diagnosed with bipolar disorder, schizophrenia, or major depression and controls were analyzed. High genetic expression of PCTK3 was found in samples from patients with schizophrenia and depression, indicating the relationship of this protein with these diseases.

7.3.2. Alzheimer's Disease

Most neurodegenerative diseases are caused by insoluble aggregates of normal cellular proteins, such as Alzheimer's Disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis [81].

AD is caused by a lesion in the extracellular amyloid plaque, in which aggregated Aβ peptide accumulates before the onset of disease symptoms, represented by dementia, memory deficits, and executive function [99]. The amyloid β (Aβ) peptide accumulated in AD can be composed of oligomeric structures without structural definition, or it can become truncated, pyroglutinated, and phosphorylated [100]. The β-secretase and γ-secretase enzymes are responsible for the cleavage of the amyloid precursor protein (APP) forming the Aβ peptide [101]. The formation of different APP fragments, each with different cellular functions, makes it difficult to determine how each metabolite influences AD [99].

The role of CDKs in AD is related to abnormal phosphorylation in lesions of the neurofibrillary tangle, the biochemical event that correlates most clinically to the disease pathology [102].

Judge *et al.* [103] reported a marked expression of cell cycle regulatory proteins, along with increased APP phosphorylation at Thr668, in mouse models of AD.

Chaput *et al.* [104] carried out a study with B103 rat neuroblastoma cells that are null for or expressing the APP-695 (B103-695) isoform. The researchers found out that APP expression promotes signaling pathways that can be related to neuronal degeneration caused by the cell cycle, verified in AD [104].

The vicious cycle of neurodegeneration in AD is caused by the induction of entry into the neuronal cell cycle by Aβ; which favors the APP phosphorylation process and β-secretase-mediated proteolysis, favoring the formation of more Aβ [99].

PCTK3 expression increased slightly in transgenic PS/APP mice. PCTKs 2 and 3 were located in the dense amyloid plaques, through immunostaining analyses of the Tg-AD mouse brain slices, which indicates the participation of these proteins in the AD [99].

Marked levels of PCTK3 in the temporal cortex of brains with AD were also identified by researchers Herskovits and Davies (2006) [48]. These data indicate the indirect involvement of PCTK3 in the phosphorylation of Tau at residues T231 and S235, and in the promotion of AD.

Although further studies are needed to fully understand the development of AD neurodegeneration and pathology, based on the results of Chaput *et al.* [99], it is possible to verify the relevance of increased expression or post-modification changes of PCTK3 in the pathogenesis of AD.

7.3.3. Demyelinating Diseases

Demyelinating diseases occur in the brain or spinal cord due to inflammation in the myelin sheath of the nerves. Demyelination is characterized by the destruction of normal myelin with elective preservation of axons. Myelin inflammation can be secondary to another cause (vaccine, infection, or environmental factors), or autoimmune processes, impairing the conduction of signals on the affected nerves [105].

Table 1. List of activities of PCKT3 and how they are linked to diseases.

Activity	Relationship with Diseases	Refs.
Binding partner with 14-3-3 protein family	14-3-3 binding partner has been localized in neurofibrillary tangles in Alzheimer's Disease (AD) brain.	[65, 66]
Modulation of cargo transport in membrane traffic	PCTK3 depletion by RNAi led to changes in the function and organization of the early secretory pathway.	[62]
PCTK3 was identified as a novel p53 responsive gene capable of inducing cell cycle arrest and cell death	The p53 protein acts in controlling cell division and death. p53 gene is considered a tumor suppressor gene, therefore mutations in its DNA sequence can cause cells to grow and spread. More than 50% of human cancers have mutations that lead to loss of p53 function.	[63, 64]
PCTK3 indirectly stimulates phosphorylation of Tau at Thr231 and Ser235	Tau protein is known to stabilize the abundant microtubules in neurons and when defective, can lead to dementia states like AD. The study of paired helical filaments, isolated from brain tissues of patients with AD, detected a high concentration of PCTK3. Thr231 and Ser235 sites are residues that are modified early in the AD pathogenesis process.	[48]
Genome integrity regulator, through interaction with RAD9, RAD17, and TOPBP1.	The depletion of PCKT3 causes chromosomal abnormalities and an increase in endogenous DNA damage.	[67]
Regulation of cell migration and adhesion, controlling the dynamics between the activities of RhoA, Rac1, and ROCK proteins.	PCTK3 knockdown increased the phosphorylation of cofilin at Ser312 and led to an increase in cell motility and kinase activity associated with Rho. Cellular motility mechanisms are associated with various physiological phenomena including metastasis in malignant cancers.	[68]
PCTK3 promotes oligodendrocyte precursor cells (OPC) differentiation.	PCTK3 was mainly expressed in differentiated oligodendrocytes. Differentiation of OPCs is related to demyelinating diseases.	[70]
PCTK3 was identified as an APP modifier in transgenic *Drosophila* and its human homologs.	Most neurodegenerative diseases are caused by insoluble aggregates of normal cellular proteins, such as AD, Parkinson's disease, and amyotrophic lateral sclerosis.	[74]
PCTK3 is required for the cAMP-induced redistribution of AQP2. PCTK3 phosphorylates Ser261 of the AQP2	PCTK3 can control AQP2. Many diseases such as liver cirrhosis and diabetes insipidus, which are associated with unregulated AQP2, can be favored by controlling this protein complex.	[71]

Demyelinating diseases impair sensation, locomotion, cognition, and other functions, depending on the areas and nerves involved. They include, among others, multiple sclerosis (MS), optic neuromyelitis (Dévic's disease), acute disseminated encephalomyelitis (ADEM), and acute hemorrhagic leukoencephalopathy (AHL) [105].

Expression analyses showed the deregulation of genes associated with inflammation, cell death, DNA damage, and DNA repair, p53 function, and genes associated with tissue repair, RNA metabolism, and regulation of transcription or translation [77].

Abnormal myelination and remyelination processes are associated with the differentiation of OPCs. Guo *et al.* verified through RNA sequencing a high expression of PCTK3 in oligodendrocytes. PCTK3 is highly expressed in specific regions, such as the heart, spinal cord, and brain, which make this protein a potential target for drug development related to demyelinating diseases [77].

7.3.4. Cerebral Ischemia

Cerebral ischemia (CI) is among the leading causes of death and sequelae worldwide. CI is the most frequent type of stroke, and has two classifications: the first is focal ischemia, in which a clot obstructs a vessel and reduces the passage of blood to the brain, causing the death of cells in that region; the second is global ischemia, in which the entire irrigation is compromised, causing potentially more severe damage [106].

CI causes delayed neuronal death in the hippocampal CA1 region [107]. The hippocampus region is necessary for memory and is susceptible to ischemia-reperfusion [107]. Occasionally cognitive deficits and delayed neuronal death are often a consequence of CI; processes that are closely related to neural plasticity in the hippocampus CA1 region [108].

Wang *et al.* [109] evaluated the expression of proteins in the hippocampus region of rats, after treatment with Xiao-Xu-Ming (XXM) extract with two vessel occlusions (2-VO). Through unmarked quantitative proteomics techniques, the molecular events associated with chronic cerebral hypoperfusion and XXM extract modulation were investigated. The results showed that 52 proteins were expressed differentially, which are associated with various molecular functions and biological processes, including PCTK3.

The main activities of PCTK3 discovered so far and their relationships with several diseases are summarized in Table **1**.

CONCLUSION

Although a growing interest in CDKs has emerged since the discovery of their roles in the cell cycle and transcription, the PCTAIRE family has not been extensively studied to date, and PCTK3 is the least investigated among these family members. It is known that PCTK3 has two isoforms, is expressed in many tissues (especially in the brain and heart), and can be fully activated either by binding to a cyclin or by PKA phosphorylation (a feature that is not common in the CDK family).

Different from most of the other CDKs, PCTK3 is involved in several biological events in the brain. Its deregulation is directly related to many neurological diseases, such as depression, Alzheimer's disease, demyelinating diseases, and cerebral ischemia. Therefore, special attention should be given to this protein as a potential target for drugs in treating these diseases.

The studies mentioned in this review demonstrate how diverse are PCTK3 activities and their relations with diseases. However, even though these researches show very relevant information, there is still a lot to investigate about the PCTK3 structure, its biophysical/biochemical aspects, how this enzyme acts and is recruited for biological events. From a more in-depth knowledge about PCTK3, it will be easier to develop new methods for early diagnosis and/or therapies in which control of PCTK3 activity results in a better treatment for the related diseases.

LIST OF ABBREVIATIONS

14-3-3	=	Proteins Located in the 14th Fraction Eluting from a DEAE-cellulose Column and in Position 3.3 on a Starch Electrophoresis Gel
9-1-1	=	Heterotrimeric Rad9-Rad1-HUS1
AD	=	Alzheimer's Disease
ADP	=	Adenosine Diphosphate
AKAP	=	A-kinase Anchoring Protein
APP	=	Amyloid β-precursor Protein
AQP2	=	Aquaporin-2
ATP	=	Adenosine Triphosphate
ATR	=	Ataxia Telangiectasia and Rad3-related Kinase
ATRIP	=	ATR-interacting Protein
AVP	=	Arginine-vasopressin

cAMP = Cyclic Adenosine Monophosphate
cdc2 = Cell Division Cycle Protein 2
CDC25A = Cell Division Cycle 25 Homolog A
CDC25C = Cell Division cycle 25 Homolog C
CDK = Cyclin-dependent Kinase
Chk1 = Checkpoint Kinase 1
CHO = Chinese Hamster Ovary
COPII Complex = Coat Protein Complex II
CTS-1 = p53-derived Synthetic Tumor Suppressor
CXXC4 = CXXC Finger Protein 4
DDR = DNA Damage Response (DDR)
DNA = Deoxyribonucleic Acid
dsDNA = Double Strand DNA
ELK1 = ETS Domain-containing Protein-1
ERK = Extracellular Signal-regulated Kinases
FAK = Focal Adhesion Kinase
FDA = Food and Drug Administration
GBM = Glioblastoma
GDP = Guanosine-5'-diphosphate
GSC = Glioblastoma Stem-like Cells
GTP = Guanosine-5'-triphosphate
HR = Homologous Recombination
MAPK = Mitogen-Activated Protein Kinase
MEK = Mitogen-activated Protein Kinase Kinase
MLC = Myosin-Light-Chain Kinase
mRNA = Messenger RNA
OPC = Oligodendrocyte Precursor Cells
p53 = Tumor Protein p53
PARP = Poly(ADP-ribose) Polymerase
PARPi = PARP Inhibitors
PCR = Polymerase Chain Reaction
PCTK1 = PCTAIRE 1
PCTK2 = PCTAIRE 2
PCTK3 = PCTAIRE 3
PCTKs = PCTAIRE Kinases
PDB = Protein Data Bank
PKA = Protein Kinase A
PLCβ1 = phospholipase Cβ 1
qRT-PCR = Real-time Quantitative Reverse Transcription PCR
RAC1 = Ras-related C3 Botulinum Toxin Substrate 1
Rad proteins = “Radiation-repair” Proteins
Raf kinase = “Rapidly Accelerated Fibrosarcoma” Kinase
RFC = Replication Factor C
RHINO = Rad9, Rad1, Hus1 Interacting Nuclear Orphan
RhoA = Ras Homolog Family Member A
RNA = Ribonucleic Acid
RNAi = RNA Interference
ROCK = Rho-associated Kinase
RPA = Replication Protein A
RTK = Receptor Tyrosine Kinase
RT-PCR = Reverse Transcription-Polymerase Chain Reaction
SH2-domain = Src Homology 2 Domain
siRNA = Small Interfering RNA
Sos Protein Family = “Son of Sevenless” Protein Family
ssDNA = Single-strand DNA
STUB1 = STIP1 Homology and U-Box Containing Protein 1
T2D = Type 2 Diabetes
Tau = T Protein
TOPBP1 = DNA Topoisomerase 2-binding Protein 1
V2R = V2 Receptors

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

Walter Figueira de Azevedo Jr. is a researcher for CNPq (Brazil) and is serving as Section Editor for the journal Current Medicinal Chemistry.

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