A Cdc2-related protein kinase hPFTAIRE1 from human brain interacting with 14-3-3 proteins

Yankun Gao¹, Mei Jiang¹, Tao Yang¹, Jian Ni¹, Jiangye Chen¹

¹State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue-yang Road, Shanghai 200031, China

hPFTAIRE1 (PFTK1), a Cdc2-related protein kinase, is highly expressed in human brain. It exhibits cytoplasmic distribution in Hela cells, although it contains two nuclear localization signals (NLSs) in its N-terminus. To search for its substrates and regulatory components, we screened a two-hybrid library by using the full-length hPFTAIRE1 as a bait. Four 14-3-3 isoforms (β , ϵ , η , τ) were identified interacting with the hPFTAIRE1. We found a putative 14-3-3 binding consensus motif (RHSSPSS) in the hPFTAIRE1, which overlapped with its second NLS. Deletion of the RHSSPSS motif or substitution of Ser¹¹⁹ with Ala in the conserved binding motif abolished the specific interaction between the hPFTAIRE1 and the 14-3-3 proteins. The mutant S120A hPFTAIRE1 also showed a weak interaction to the 14-3-3 proteins. The results suggested that the Ser¹¹⁹ is crucial for the interaction between hPFTAIRE1 and the 14-3-3 proteins. All the hPFTAIRE1 mutants distributed in cytoplasm of Hela cells and human neuroblastoma cells (SH-SY5Y) when fused to the C-terminus of a green fluorescent protein (GFP), indicating that binding with the 14-3-3 proteins does not contribute to the subcellular localization of the hPFTAIRE1, although the binding may be involved in its signaling regulation.

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Introduction

The Cdks (Cyclin-dependent kinases) are serine/threonine kinases that had been shown to be key players in the control of cell cycle progression [1]. These various Cdks share high similarity in their amino acid sequence, and contain a PSTAIRE motif for binding of their cyclin partners. Over the last few years, a number of kinases have been identified in mammalian based on structural similarity to p34^{cdc2} [2], and their cyclin partners remain to be identified. These Cdc2-related protein kinases derived their names from the presence of an amino acid substitution for serine in the cyclin binding sequence PSTAIRE. Some of these Cdc2-related kinases, such as PFTAIRE, PCTAIRE, PITSIRE and Cdk5, have been shown to play important

roles during cell division, gametogenesis and differentiation [3-6]. Two mouse *PFTAIRE* (*PFTAIRE* and *mPFTAIRE1*) genes have been reported [7, 8]. The mPFTAIRE1 has a 46 amino acid extension than the PFTAIRE at its N-terminus. The PFTAIRE is expressed primarily in the postnatal and adult nervous system by *in situ* hybridization and indirect immunofluorescence. In neurons, PFTAIRE protein was localized in the nucleus and cytoplasm of cell [7]. The *mPFTAIRE1* is ubiquitously expressed in murine tissues and highly expressed in brain, testis and embryo. The mPFTAIRE1 is implicated in the process of meiosis as well as neuron differentiation. We have isolated a Cdc2-related protein kinase PFTAIRE1 from human (hPFTAIRE1) [9]. The *hPFTAIRE1* was highly expressed in brain, pancreas, kidney, heart, testis and ovary. Although the hPFTAIRE1 contains two potential nuclear localization signals (NLS) at its N-terminus, GFP-hPFTAIRE1 fusion protein expressed in Hela cells distributed mainly in cytoplasm. To search its substrate and explore the cytoplasmic distribution mechanism, we screened an adult human brain two hybrid cDNA library by using the hPFTAIRE1 as a bait and identified

Correspondence: Jiangye Chen

Tel: 86-21-54921251; Fax: 86-21-54921011

E-mail: jychen@sunm.shnc.ac.cn

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several hPFTAIRe1 interacting proteins, including septin family member KIAA0202 [10] and four 14-3-3 isoforms (β , ϵ , η , τ).

14-3-3 proteins are a family of highly conserved acidic proteins, which are expressed ubiquitously in all eukaryotic cells, especially abundant in brain cells and other CNS cells. Seven 14-3-3 isoforms encoded by seven distinct genes are identified in mammalian cells. More than 10 isoforms are identified in plants, and two isoforms are identified in yeast, *Drosophila*, and *Caenorhabditis elegans* [11, 12]. The 14-3-3 proteins are mainly localized in cytoplasm. More and more signaling proteins have been reported as 14-3-3 ligands. This broad range of partners suggests a role for 14-3-3 as general biochemical regulators. Through interaction with its effector proteins, 14-3-3 participates in the regulation of diverse biological processes, including neuronal development, cell growth control, apoptosis, and viral and bacterial pathogenesis [13, 14]. Research work on nuclear proteins such as Cdc25 and NFAT suggests that 14-3-3 binding is also involved in intracellular targeting. 14-3-3 binding keeps these nuclear proteins in the cytoplasm [15, 16]. Many serine/threonine kinases are capable of generating 14-3-3 binding sites, including Raf-1, protein kinase A and protein kinase C. The most important feature of the 14-3-3 proteins is their capability of recognizing and binding to certain motifs containing phosphoserine. By extensive screening of phosphoserine-orientated peptide libraries, it is known that there are mainly two 14-3-3 binding consensus motifs: RSxpSxP and Rx₁₋₂Sx₂₋₃S [17]. By interaction with more than 100 binding partners, 14-3-3 proteins modulate the action of proteins that are involved in cell cycle and transcriptional control, signal transduction, intracellular trafficking and regulation of ion channels.

In this study, we screened hPFTAIRe1 interacting proteins in two hybrid system and identified four 14-3-3 isoforms (β , ϵ , η , τ). The association of the hPFTAIRe1 with 14-3-3 proteins and their intracellular targeting mechanism were analyzed.

Materials and Methods

Plasmid construction

The full open reading frame of *hPFTAIRe1* gene was inserted into *EcoRI* and *BamHI* sites of pGilda vector to generate pGilda-PFT and used as a bait plasmid for two-hybrid screening. The *hPFTAIRe1* coding region was inserted into *EcoRI* and *XhoI* sites of pcDNA3-myc to construct pcDNA3-PFT-myc for co-immunoprecipitation assay. All constructed plasmids were confirmed by DNA sequencing.

Yeast two-hybrid assay

A human adult brain LexA two-hybrid cDNA library (Clontech) in which cDNA fragments inserted in pB42AD was used for two-hybrid screening. pGilda-PFT (LexA-PFTAIRe1 fusion) was used as a bait to screen the library. All the procedures were followed the

protocol from Clontech.

The pGilda-PFT and library plasmids were sequentially transformed into yeast host strain CJY151 (*MATa*, *his3*, *trp1*, *LexA_{op(Δ6)}-leu2*, *LexA_{op(Δ8)}-lacZ*) [18]. A total of 1×10^8 transformants were plated onto the yeast galactose drop-out minimal medium lacking of histidine, tryptophan and leucine, incubated for 3 days at 30 °C. The Leu⁺ colonies were collected and assayed for β -galactosidase activity. The library plasmids from the positive colonies (Leu⁺ and β -Gal⁺) were confirmed by PCR based restriction map and DNA sequence analysis.

Mutagenesis and β -galactosidase activity assay

Three hPFTAIRe1 mutants: hPFTAIRe1D (hPFTAIRe1 with S¹¹⁹SPSS 5 residues deleted), S119AhPFTAIRe1 (hPFTAIRe1 with Ser119→Ala substitution) and S120AhPFTAIRe1 (hPFTAIRe1 with Ser120→Ala substitution) were constructed by PCR amplification using Mutagenesis kit (Stratagene). The hPFTAIRe1 mutants were constructed into pGilda, pcDNA3 and pEGFPC2. All plasmids were confirmed by sequencing. Various pairs of the pGilda-PFT mutants and pB42AD-14-3-3 plasmids were cotransformed into yeast reporter strain CJY151 (*MATa*, *his3*, *trp1*, *LexA_{op(Δ6)}-leu2*, *LexA_{op(Δ8)}-lacZ*) [18] to test for the β -galactosidase activity. β -galactosidase assays with X-gal as substrate was performed as described (MATCHMAKER Two-Hybrid System, Clontech).

Co-immunoprecipitation assay and Western blot analysis

Hela cell extracts were prepared by homogenizing cultured cells in CelLytic M cell lysis reagent (Sigma). Insoluble materials were pelleted by centrifugation at $12\,000 \times g$ for 15 min at 4 °C. Protein concentration was determined using a Bio-Rad DC protein assay kit (Bio-Rad). Western blot analysis was conducted the same as previously described [18]. Appropriate antibodies were used to detect the corresponding proteins according to Sambrook [19]. The anti-HA, anti-LexA, anti-myc, anti-14-3-3 β and anti-GFP antibodies were purchased from Santa cruz. The mouse IgG was purchased from Sigma. The hPFTAIRe1 was expressed and purified from bacteria (data not shown). The rabbit polyclonal anti-PFTAIRe1 antibody was made by antibody research center in Shanghai Institutes for Biological Sciences, CAS.

Subcellular localization

Hela cells and SH-SY5 cells were seeded on coverslips and transiently transfected with 5 μ g of pEGFP-C2 (Clontech), pEGFPC2-PFT, pEGFPC2-PFTD, pEGFPC2-S119APFT and pEGFPC2-S120APFT respectively using Lipofectamine reagent (Gibco BRL). 48 hours after transfection, cells were washed three times with PBS, fixed 20 min in 4% paraformaldehyde, washed three times again with PBS. To visualize nuclei, cells were treated with 0.1% triton X-100 and incubated in 1 mg/ml 4, 6-diamidino-2-phenylindole (DAPI) in phosphate-buffered saline.

Results

Interactions between the hPFTAIRe1 and four 14-3-3 isoforms (β , ϵ , η , τ)

The *hPFTAIRe1* encodes for a Cdc2-related protein kinase that is highly expressed in human brain [9]. To search for its regulatory substrates an adult human brain

Lex-A two-hybrid library was used to screen for its interacting proteins. A full-length hPFTAIRES1 was used as a bait. Among 1×10^8 colonies screened, 243 colonies could grow on the minimal medium, 81 of them showed a positive response in the β -galactosidase assay. These 81 inserts represent 7 different cDNA fragments, 4 of them encoding for four 14-3-3 isoforms (β , ϵ , η , τ). All the cDNA inserts from pB42AD-14-3-3 plasmids encoded for full open reading frame of 14-3-3 proteins which fused with 88-residues acidic activator B42 and a hemagglutinin (HA) epitope tag in frame. The cDNA inserts for 14-3-3 β , 14-3-3 ϵ and 14-3-3 τ are 1 kb in length and that for 14-3-3 η is 2 kb which contains a longer 3'-non coding region.

The interactions between hPFTAIRES1 and the four 14-3-3 isoforms were verified by two hybrid analysis. Co-transforming pGilda-PFT and pB42AD-14-3-3 plasmids into the yeast CJY151 showed β -galactosidase activity in 3 h incubation at 30 °C (Figure 1A). The pGilda-PFT or pB42AD-14-3-3 plasmids co-transformed with control

vectors did not detect any β -galactosidase activity. The interaction between the hPFTAIRES1 and the four 14-3-3 isoforms can also promote the expression of *LEU2* gene when it is used as a reporter, all the co-transformed strains grow well in leu⁻ medium (Figure 1B). The physical interactions between the hPFTAIRES1 and the four 14-3-3 isoforms were confirmed by co-immunoprecipitation in yeast cells. LexA-hPFTAIRES1 was co-expressed separately with four HA-14-3-3 proteins in CJY151. The yeast lysates were immunoprecipitated by anti-LexA antibody and then detected with anti-HA antibody using Western blot. A 35 kDa band which corresponds to 14-3-3 was detected in the pGilda-PFT and pB42AD-14-3-3 co-transformed cells, and the control showed a negative result (Figure 1C).

Since the 14-3-3 proteins are ubiquitously expressed in all eukaryotic cells and hPFTAIRES1 is also expressed in several cell lines tested (data not shown). Therefore Hela cells were used to confirm the interaction between hPFTAIRES1 and 14-3-3 proteins *in vivo*. The immuno-

Figure 1 Interaction of hPFTAIRES1 with four 14-3-3 isoforms (β , ϵ , η , τ). **(A)** β -galactosidase activity assay. The pGilda-PFT and pB42AD-14-3-3 plasmids were co-transformed in CJY151. pGilda-Positive or pB42AD was used as a control. **(B)** Growth ability of transformants on minimal medium deficient in His, Trp and Leu. **(C)** Co-immunoprecipitation assay. The LexA-PFTAIRES1 and the HA-14-3-3 proteins were co-expressed in CJY151. The expression of LexA-PFTAIRES1 was showed as above by using anti-LexA antibody in Western blot analysis. The lower panel showed the interaction between hPFTAIRES1 and 14-3-3 proteins in yeast. The tagged proteins were pulled down with anti-LexA antibody and detected in Western blot with anti-HA antibody as a probe. **(D)** Interaction of the hPFTAIRES1 with 14-3-3 proteins in Hela cells. The 14-3-3 proteins in Hela cells were pulled down with mouse monoclonal anti-14-3-3 β antibody. The hPFTAIRES1 was immunoprecipitated with polyclonal anti-PFTAIRES1 antibody. The lysate was immunoprecipitated with mouse IgG and used as a control. The precipitated proteins were analysed by Western blot using anti-14-3-3 β antibody.

precipitations of hPFTAIRES1-14-3-3 complexes were performed with anti-PFTAIRES1 or anti-14-3-3 β antibody. The precipitated proteins were detected and analyzed by Western blotting with anti-14-3-3 β antibody. The 14-3-3 proteins were pulled down and detected in cell lysate (Figure 1D, first lane), and the hPFTAIRES1 could be pulled down and detected to bind with 14-3-3 proteins (Figure 1D, second lane). The results suggested that the hPFTAIRES1 could interact with the 14-3-3 proteins *in vivo*.

The hPFTAIRES1 RHSSPSS motif is required for interaction with 14-3-3 proteins

Ligands of 14-3-3 are known to contain defined interaction motifs including two main binding consensus motifs: RSxpSxP and Rx₁₋₂Sx₂₋₃S. The hPFTAIRES1 contains a ~140 aa N-terminal domain, a ~300 aa kinase conserved domain in its central region, and a ~30 aa C-terminal domain. The N-terminal region contains two predicted nuclear localization signals (NLSs) located at residues 66 to 72 (PEDKKVR) and 113 to 119 (PKVRRHS). A putative 14-3-3 binding consensus motif RHSSPSS (117 to 120) was overlapped with the second NLS of the hPFTAIRES1 (Figure 2A).

To examine whether the putative consensus motif in the hPFTAIRES1 is required for binding of the 14-3-3 proteins,

5 residues of SSPSS from the motif was deleted by PCR based mutagenesis strategy. The fragment was inserted into vectors to produce mutated hPFTAIRES1 fusion proteins for further binding analysis. The pGilda-PFT Δ was introduced into CJY151 and the LexA-hPFTAIRES1 Δ was co-expressed with the four 14-3-3 isoforms in yeast cells and assayed in two hybrid system. The β -galactosidase activity and Leu⁻ medium growth ability analysis revealed that the hPFTAIRES1 mutant failed to interact with all four 14-3-3 isoforms (Figure 3A and 3B). The pcDNA3-PFT Δ -myc was transfected into Hela cells to detect the interaction between hPFTAIRES1 Δ and 14-3-3 proteins. The pcDNA3-PFT-myc was also transfected into Hela cells and used as a positive control (Figure 3C left panel). Co-IP and Western blot analysis showed that the interactions between the hPFTAIRES1 and 14-3-3 proteins were abolished by deletion of the 14-3-3 binding motif in the hPFTAIRES1 (Figure 3C right panel). The results indicate that the putative motif RHSSPSS in the hPFTAIRES1 is required for its interaction with 14-3-3 proteins.

The Ser¹¹⁹ is crucial for the interaction between hPFTAIRES1 and 14-3-3 proteins

The 14-3-3 proteins bind to specific phosphoserine con-

Figure 2 Mutagenesis strategy. The hPFTAIRES1 was drawn schematically, two predicted nuclear localization signals (NLSs) were indicated in black box and conserved kinase domain was shaded. In region of 66-131aa, the shaded amino acids indicate the two NLSs, and the boxed amino acids represent a putative 14-3-3 binding consensus sequence which overlapped with the second NLS. 5 residues of SSPSS in the 14-3-3 binding motif were deleted by using a mutagenesis kit (A). Residue of Ser¹¹⁹ or Ser¹²⁰ in the motif was substituted with Ala respectively (B).

Figure 3 Examination of interaction between hPFTAIRES1 Δ and 14-3-3 proteins. **(A)** The pGilda-PFT Δ and pB42AD-14-3-3 plasmids were co-transformed into CJY151, co-expressed LexA-PFTAIRES1 Δ and HA-B42-14-3-3s were assayed for β -galactosidase activity. pGilda-Positive, pGilda-PFT or pB42AD was used as a control. **(B)** Growth ability of the transformants on minimal medium. **(C)** Interaction of hPFTAIRES1 Δ with 14-3-3 proteins in Hela cells. The myc tagged hPFTAIRES1 Δ was immunoprecipitated from Hela cell lysates and analysed by Western blot using anti-myc or anti-14-3-3 β antibody. Myc tagged hPFTAIRES1 was used as a positive control.

Figure 4 Interaction of S119A or S120A hPFTAIRES1 mutants with 14-3-3 proteins in yeast two hybrid system. The pGilda-S119APFT and pB42AD-14-3-3 plasmids were co-transformed into CJY151. Co-expressed LexA-S119APFTAIRES1 and HA-B42-14-3-3 were assayed in β -galactosidase activity **(A)** and Growth ability on minimal medium **(B)**. LexA-S120APFTAIRES1 and HA-B42-14-3-3 were co-expressed in CJY151 for binding assay. The β -galactosidase activity **(C)** and growth ability of the transformants on minimal medium **(D)** were examined. pGilda-Positive, pGilda-PFT or pB42AD were used as controls.

Figure 5 Interaction of hPFTAIRE1 mutants with 14-3-3 proteins in Hela cells. **(A)** GFP tagged hPFTAIRE1 or hPFTAIRE1 mutants were determined by using anti-GFP antibody in Western blot analysis. **(B)** The expression level of endogenous 14-3-3 proteins was determined with anti-14-3-3β antibody. **(C)** The 14-3-3 proteins were immunoprecipitated from the lysates and analysed by Western blot using anti-GFP antibody. **(D)** The GFP tagged hPFTAIRE1 and hPFTAIRE1 mutants were immunoprecipitated from the lysates and detected with anti-14-3-3β antibody.

taining motifs and their dimeric nature allows them to act as intra- and intermolecular bridges, effectively colocalizing proteins in a phosphorylation dependent manner [20]. To exam whether the interaction between hPFTAIRE1 and 14-3-3 proteins is phosphorylation dependent, point mutations were introduced into the 14-3-3 binding motif in the hPFTAIRE1 by PCR based mutagenesis strategy (Figure 2B). Two hPFTAIRE1 mutated fragments were inserted into vector to produce pGilda-S119APFT and pGilda-S120APFT for two hybrid binding assay. The β-galactosidase activity and Leu⁻ medium growth ability analysis revealed that the S119AhPFTAIRE1 failed to interact with all four 14-3-3 isoforms (Figure 4A and 4B), however, S120AhPFTAIRE1 still could interact with 14-3-3 isoforms

though the interaction was weaker than the wild type form (Figure 4C and 4D). The pEGFPC2-S119APFT and pEGFPC2-S120APFT were introduced into Hela cells to detect the interaction of two hPFTAIRE1 mutants with 14-3-3 proteins by Co-IP and Western blotting. Consistent with the two hybrid result, the mutation in Ser¹¹⁹ abolished the interaction between the hPFTAIRE1 and 14-3-3 proteins, but the S120A hPFTAIRE1 mutant showed a weak interaction with the 14-3-3 proteins (Figure 5C and 5D). The results indicate that both Ser¹¹⁹ and Ser¹²⁰ in the hPFTAIRE1 contribute to its binding with 14-3-3 proteins, and the Ser¹¹⁹ is crucial for the interaction between them. It is possible that the interaction between hPFTAIRE1 and 14-3-3 protein may depend on phosphorylation of the Ser¹¹⁹.

Binding to 14-3-3 does not regulate the hPFTAIRE1 intracellular localization

The hPFTAIRE1 contains two predicted NLSs in its N-terminus, 184-197 (LLKGLKHANIVLLH) and 235-245 (LFLFQLLRGLS) (Figure 2A). Lazzaro *et al* reported that mouse PFTAIRE protein was localized both in nucleus and cytoplasm in neurons by indirect immunofluorescence. Our previous studies showed that the GFP-hPFTAIRE1 fusion was mainly localized in the cytoplasm of Hela cells [9]. 14-3-3 proteins are implicated in regulating the function and subcellular localization of numerous signaling molecules through binding to their partners. We hypothesized that the binding of 14-3-3 proteins may be involved in regulating hPFTAIRE1 subcellular distribution.

We transfected pEGFPC2-PFTΔ, pEGFPC2-S119APFT and pEGFPC2-S120APFT into Hela cells and SH-SY5Y cells, pEGFPC2-PFT and pEGFPC2 were used as controls. Similar to GFP-PFTAIRE1, the ectopically expressed GFP-PFTAIRE1Δ, GFP-S119APFTAIRE1 and GFP-S120APFTAIRE1 were mainly localized in cytoplasm while GFP protein distributed everywhere in Hela cells and in SH-SY5Y cells (Figure 6A and 6B). To verify that full-length instead of partial GFP- fusion protein was expressed in Hela cells, lysate from the transfected cells were immunoblotted with anti-GFP antibody. It was showed that all the expressed fusion proteins were in predicted size (Figure 5A). Deletion or point mutations in the 14-3-3 binding motif sequence did not lead the hPFTAIRE1 into nucleus indicating that binding with 14-3-3 is not required for its regulation of intracellular localization.

Discussion

The hPFTAIRE1 belongs to a member of cyclin-dependent kinase (cdk) family whose function is unknown. The kinase activity in the PFTAIRE immune complex, precipitated from a mouse brain cytosolic protein prepara-

Figure 6 Subcellular localization of hPFTAIRES1 and hPFTAIRES1 mutants. **(A)** HeLa cells were transfected with pEGFPC2, pEGFPC2-PFT, pEGFPC2-PFTΔ, pEGFPC2-S119APFT and pEGFPC2-S120APFT respectively. **(B)** The plasmids were introduced into SH-SY5Y cells. Green fluorescence of GFP, GFP-hPFTAIRES1, GFP-hPFTAIRES1Δ, GFP-S119AhPFTAIRES1 and GFP-S120AhPFTAIRES1 were observed under a fluorescence microscope (left panels), the nuclei were stained with DAPI (right panels).

tion, phosphorylated two specific sets of proteins of ~58-60 and 200-205 kDa. These proteins remained associated with PFTAIRES during the immunoprecipitation [7]. The immunoprecipitated PFTAIRES was not able to phosphorylate histone H1, NF-H and other common substrates, suggesting that the PFTAIRES may have a more restricted set of substrates than other cdks. *In vitro*-translated PFTAIRES or bacteria produced GST-PFTAIRES did not have kinase activity on their own (autophosphorylation). It is conceivable that these preparations of the PFTAIRES lacked regulatory components that may be required for its kinase activity. We identified several hPFTAIRES1 interacting proteins through two-hybrid screening. One of them we reported previously was a human septin member encoded by KIAA0202 [10]. The KIAA0202 is a 508 aa protein and has a predicted molecular mass of 58 kDa which has similar size with the PFTAIRES substrates of 58-60 kDa *in vivo*. The cytoplasmic localization of septins suggested that the KIAA0202 might act as a localizer or specific substrate for the hPFTAIRES1. In this study, we reported that four 14-3-3 isoforms (β , ϵ , η , τ) are able to interact with the hPFTAIRES1, suggesting a novel regulatory mechanism for hPFTAIRES1 may be

involved. Binding with 14-3-3 proteins is involved in some nuclear proteins intracellular targeting, in case of Cdc25 and NFAT, 14-3-3 binding keeps them in the cytoplasm. It is also reported that binding of 14-3-3 to TTP is one of multiple mechanisms that keep TTP in the cytoplasm [22, 23]. The hPFTAIRES1 contains a putative 14-3-3 binding motif RHSSPSS overlapped with its second NLS. Deletion of 14-3-3 binding motif blocked the interaction. This result suggested that hPFTAIRES1 binds 14-3-3 proteins through its 14-3-3 binding motif RHSSPSS. It was also reported that this binding is in a phosphorylation dependent manner. Among the two binding consensus motifs: RSxpSxP and Rx1-2Sx2-3S, the phosphorylation of one Ser is crucial for the interaction. Our results showed that substitution of Ser¹²⁰ with Ala weaken the interaction, while hPFTAIRES1 Ser¹¹⁹Ala completely lose its ability to interact with 14-3-3 proteins reflecting that phosphorylation on Ser¹¹⁹ may be required for the binding. All the mutants including hPFTAIRES1Δ, S119APFTAIRES1 and S120APFTAIRES1 showed a similar cytoplasmic distribution like the wild type form of the hPFTAIRES1 both in neuron cells and HeLa cells, indicating that 14-3-3 binding is not required

for intracellular trafficking of the hPFTAIRE1. Interacting with the KIAA0202 support the suggestion of that the hPFTAIRE1 may play roles in cytoplasm. PCTAIRE1, another Cdc2-related kinase member also exhibits cytoplasmic distribution throughout the cell cycle [24]. Although our data showed that the ectopically expressed hPFTAIRE1 is mainly located in cytoplasm in neuron cells and Hela cells, mPFTAIRE1 was reported to be distributed both in nucleus and cytoplasm in neurons. Many proteins with nuclear localization signal (NLS) can be imported into nucleus mediated by importin α/β complex. Proteins containing canonical leucine-rich nuclear export signal (NES) were recognized by some export receptor complex (exportin1 or Crm1) and exported to cytoplasm [21]. A protein that shuttles between nucleus and cytoplasm usually contains a NLS that binds to an importin and a NES that binds to an exportin. The mechanism for the hPFTAIRE1 shuttling between cytoplasm and nucleus need to be further investigated.

The majority of the cells in brain and testis are not active in mitosis. The expression pattern of the hPFTAIRE1 predominantly in the brain and testis suggested that the function of the hPFTAIRE1 is different from the function of the classic Cdk family proteins that are essential for the control of mitotic cell cycle. It is also known that 14-3-3 proteins are abundantly expressed in the brain. 14-3-3 proteins have attracted much interest recently owing to their possible involvement in the pathophysiology of various neurological disorders. Mutations in some 14-3-3 genes lead to disruption of neuronal differentiation and synaptic plasticity in *Drosophila*. In humans, an interesting observation is the detection of significantly decreased levels of 14-3-3 γ in the cortex of embryos with Down's syndrome, which may related to the impairment of neuronal differentiation, synaptic plasticity and signalling pathways. Important influence on neuronal migration has also been reported for 14-3-3 ϵ . Binding of 14-3-3 ϵ to Cdk5/p35-phosphorylated Nudel maintains Nudel phosphorylation by protecting it from dephosphorylation by the serine/threonine phosphatase PP2A. This binding has positive effects on the motor function of dynein and therefore on neuronal migration [25]. It will be interesting to explore whether hPFTAIRE1 is involved in the neuron differentiation and migration.

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