東北大学機関リポジトリ
TOUR
Tohoku University Repository

Novel Functions of Prolyl Isomerase Pin1(Recent Topics of the Agricultunal Biological Science in Tohoku University)

著者	AKIYAMA Hirotada, TATARA Yota, UCHIDA Takafumi
journal or publication title	Tohoku journal of agricultural research
volume	58
number	3/4
page range	147-156
year	2008-03
URL	http://hdl.handle.net/10097/40439

Tohoku Journal of Agricultural Research
Vol. 58 No. 3-4, March 2008
Printed in Japan

Novel Functions of Prolyl Isomerase Pin1

Hirotada AKIYAMA, Yota TATARA and Takafumi UCHIDA*

Molecular Enzymology, Molecular Cellular Biology, Agricultural Science, Tohoku University

(Received, January 11, 2008)

Abstract

We have studied the novel functions of peptidyl prolyl *cis/trans* isomerase Pin1 that specifically binds the phosphorylated Ser/Thr-Pro protein motifs and catalyzes the *cis/trans* isomerization of the peptide bond. Accumulating studies have revealed that Pin1 isomerase activity is regulated by its post-translational modifications, including phosphorylation and oxidation. Various transcription factors and regulators have been identified as substrates for Pin1. It enhances AP-1 activity via isomerization of both c-Jun and c-Fos for cellular proliferation and stabilizes the oncosuppressors p53 and p73 against DNA damage at the checkpoint. We demonstrated the association between the intracellular form of Notch1 (NIC) and Pin1 by analyzing Pin1/p53 double knockout mice. Pin1 also regulates the posttranscriptional level of some cytokines, associated with asthma, that possess 3′ untranslated region AU-rich elements (ARE) via interaction with AUF-1, the nucleoprotein in the ARE-binding complex. Pin1 has been identified as the molecular partner of tau and amyloid precursor protein (APP), the key factors of Alzheimer's disease (AD). It interacts with the phosphorylated Thr-231 of tau and regulates its activity to bind microtubules. It further interacts with the phosphorylated Thr-668 of APP and affects its metabolism. Thus, Pin1 is probably involved in the pathogenesis of human pathologies, including cancer, asthma and AD, presenting an attractive target for future therapeutical drugs.

Novel Functions of Prolyl Isomerase Pin1

Cancer

Pin1 belongs to the parvulin family of PPIases and is inhibited neither by cyclosporin A nor by FK506. Among the PPIases, Pin1 is the only enzyme known to be highly specific for substrates with phosphorylated Ser or Thr, followed by a Pro, and it is structurally and functionally related to Ess1/Ptf1, which is an essential protein in budding yeast. Pin1 consists of the PPIase domain and group IV WW domains, and it specifically associates with a phosphor-

*correspondence

ylated Ser or Thr residue, followed by a Pro residue (pSer/pThr-Pro) in order to catalyze both *in vivo* and *in vitro cis-trans* isomerization of the peptidyl prolyl bonds in peptides and proteins. Interestingly, both the PPIase and WW domains recognize the pSer/pThr-Pro motifs Pinl interacts with its substrates via recognition of the specific phosphorylated Ser/Thr-Pro bond and promotes conformational changes by facilitating the *cis-trans* isomerization of these peptide bonds. Pinl substrates might represent target proteins, including mitosis-specifc phosphoproteins, for Pro-directed protein kinases such as those belonging to the MAPK and CDK family.

Among Pinl substrates, RNA polymerase II is responsible for transcription of mRNA-encoding genes, and takes part in posttranscriptional events. The C-terminal domain of the RNAP II is essential for cell viability and gene transcription. The CTD can be extensively phosphorylated, and undergoes dynamic changes in phosphorylation during the transcriptional cycle. Pinl influences the phosphorylation status of the CTD by inhibiting the CTD phosphatase and stimulating CTD phosphorylation by cdc2/cyclinB. Hyper-hyperphosphorylated form of RNAP II accumulates in M-phase cells in Pinl-dependent manner. Including CTD of RNAP II, the elements of the transcription machinery is one of the key targets of Pinl.

Isomerization by Pinl modulates protein folding, biological activity, stability, and subcellular localization of its substrates and also regulates the factors involved in the control of cell growth and apoptosis. Depletion of Pinl/Essl from yeast or HeLa cells induces mitotic arrest, whereas HeLa cells that overexpress Pinl are arrested in the G2 phase of the cell cycle. Hence, Pinl is an essential PPIase that presumably regulates mitosis by interacting with the mitotic phosphorylated proteins (MPM-2 antigens), including Cdc25, NIMA, and Wee-1, and by attenuating its mitosis-promoting activity. Pinl-deficient mice displayed immature germ cells, mammary gland impairment, and retinal dystrophy, that is, decreased levels of cyclin Dl resulted in cyclin Dl-null phenotypes [1,2]. And when Pinl was depleted from the transgenic mice with MMTV-c-Neu/HER2/ErbB2 or -v-Ha-Ras, the rate of incidences of breast cancer in these mice were reduced. [15]. Therefore, it is revealed that Pinl physiologically control the cyclin Dl levels, and Pinl overexpression promotes tumor growth via stabilization of cyclin Dl. Furthermore, *Pin1*$^{-/-}$ mouse embryonic fibroblasts (MEF) became not to respond to serum stimulation after serum depletion-induced G0 arrest. These observations also suggest that Pinl is required for cell cycle progression subsequent to G0 arrest and mitotic progression in normal mammalian cells. Transgenic overexpression of Pinl in mammary glands induced centrosome amplification, eventually leading to mammary hyperplasia and malignant mammary tumors with overamplified centrosomes. The phenotypes of both Pinl knockout mice and Pinl overexpressing transgenic mice indicated that Pinl can

effectively induce the progression and malignancy of tumors. The molecular mechanism in Pin1 expression can be associated with tumorigenesis under the Neu/Ras signaling. The *Pin1* promoter sequence possesses neither a TATA nor a CAAT box but has 2 putative GC boxes and 3 putative E2F-binding sites. Indeed, the E2F family proteins activate the *Pin1* promoter via these E2F-binding sites. The E2F proteins also bind the *Pin1* promoter *in vitro* and *in vivo*, and increased Pin1 levels in breast cancer cell lines correlate with an increase in the binding of E2F to the *Pin1* promoter. The oncosuppressor BRCA1 represses the expression of Pin1 transcripts. Moreover, E2F overexpression enhances *Pin1* promoter activity and mRNA levels in breast cancer cells. As commonly noted in many other E2F-target genes, *Pin1* transcription and protein levels fluctuate during cell cycle progression in non-neoplastic cells.

PPIase activity of Pin1 may undergo physiological regulation in a manner specific to each organ. Recently, it was discovered that Pin1 activity is modified by phosphorylation or oxidation. These modifications might be associated with the human pathology. Lu's cohort reported that Pin1 phosphorylated by cAMP-dependent protein kinase A is an inactive form. Phosphorylation of Ser-16 in the WW domain impairs nuclear localization and inhibits its binding to substrates. The dominant-negative mutants induce a mitotic block and apoptosis and increase the number of multinucleated cells with 8N DNA content.

The *p53* tumor suppressor gene plays a complex and critical role in maintaining genome integrity. Loss of the p53 function increases susceptibility to malignant transformation, and mutations in the *p53* tumor suppressor gene are the most common genetic abnormalities in oncology that are found in >50% of all human cancers. The level of active *p53* in the cells is rapidly elevated via acetylation and phosphorylation in response to DNA damage caused by various types of stress such as UV irradiation, DNA-damaging chemicals, hypoxia, and activated oncogenes. Transcriptional activity of the *p53* gene is also physiologically elevated during organogenesis. Subsequently, the high levels of activated p53 drive transcription of a variety of genes that mediate the protein's biological functions in its decision to arrest cells for facilitating DNA repair or to destroy the cells by apoptosis. Even though some p53-deficient mice do succeed in being born, they rapidly succumb to the tumors, particularly those belonging to the thymic lymphoid lineage Further, studies have also reported on the high-frequency embryonic lethality due to female-specific developmental abnormalities, namely, neural tube-closure failure leading to exencephaly and subsequent anencephaly.

Recently, Pin1 was shown to be involved in controlling p53 accumulation and its activity in cells exposed to DNA-damaging conditions [3-4]. These reports indicated that p53 was one of the substrates for Pin1, and that the effects on p53 were the result of isomerization by Pin1. The interaction between p53

and Pin1 strictly depends on p53 phosphorylation, and it requires the p53 residues Ser-33, Thr-81, and Ser-315. On binding, Pin1 induces a conformational change in p53, enhancing its transactivation activity. The Pin1-mediated p53 activation requires the WW domain and Pin1 isomerase activity. Stabilization of p53 is impaired in UV-treated *Pin1*$^{-/-}$ cells due to its inability to efficiently dissociate from Mdm2, a regulatory partner of p53. Pin1 might influence the interaction of Mdm2 with p53, resulting in the stabilization and transactivation of p53. Pro-82 of p53 was identified to be essential for its interaction with the checkpoint kinase 2 (Chk2) and consequent phosphorylation of p53 on Ser-20, following DNA damage. These physical and functional interactions are regulated by Pin1 through cis-trans isomerization of Pro-82.

Interestingly, overexpression of the oncogenes that activate p53 promoted the interaction of p53 with recombinant Pin1. The oncogene products are required for p53-dependent caspase activation during the apoptotic responses as a result of certain DNA-damaging stresses. Hence, caspase activation during the apoptotic response might be induced by the interaction between Pin1 and p53 in the event of oncogene overexpression. In fact, we observed that the primary MEFs of both WT and *Pin1*$^{-/-}$ did not undergo apoptosis in response to DNA-damaging stimuli, including UV radiation and chemotherapeutic agents. Deficiency of p53 in the primary MEFs enhanced apoptotic cell death in response to the DNA-damaging agents. Therefore, Pin1 possibly stabilizes p53 and p73 and plays a critical role in the integration of the checkpoint induced by DNA damage.

The protein kinase CK2 participates in checkpoint control and p53 phosphorylation involved in DNA damage responses. Messenger et al. indicated that CK2 also serves as a substrate for Pin1, and that Pin1 influences CK2 stabilization. The effects of Pin1 on the phosphorylation of recombinant p53 by CK2, following UV stimulation. With regard to the human osteosarcoma cell line U2-OS, UV radiation failed to enhance CK2 phosphorylation, and Pin1 did not exhibit any effect on the ability of CK2 to phosphorylate p53 ; however, it is important that the interactions between p53 and Pin1 are phosphorylation-directed. Hence, Pin1 may influence the CK2-catalyzed phosphorylation of p53 when p53 is phosphorylated in a manner that promotes Pin1 interaction. Messenger et al. reported that Pin1 inhibited the phosphorylation activity of CK2 toward topoisomerase IIα, the phosphorylation of which is essential for the G2/M transition. Hence, topoisomerase IIα might undergo phosphorylation to facilitate progression of G2/M transition by *Pin1* deficiency. The topoisomerase II inhibitor etoposide influenced the Pin1-deficient MEFs to a lesser degree than WT MEFs in response to G1 arrest. The interaction of Pin1 with p53 might depend on the topoisomerase II-CK2 system with regard to cellular growth or checkpoint control.

Further, since we have been interested in the physiological link between Pin1

and p53, we recently generated *Pin1*$^{-/-}$*p53*$^{-/-}$ mice. Pin1 knockout in mice completely prevented malignant tumor development induced by p53 knockout. Although these mice could develop, the proportion of males (80%) was significantly higher than that of females (20%). The *Pin1*$^{-/-}$*p53*$^{-/-}$ mice (median survival time ($t_{1/2}$) of 141 days) died earlier than the p53$^{-/-}$ mice ($t_{1/2}$ of 195 days) and the immature thymocytes, i.e., the CD4-CD8- (double negative) cells, displayed thymic hyperplasia. The thymocytes obtained from the *Pin1*$^{-/-}$ *p53*$^{-/-}$ mice possessed a substantial amount of NIC1, and both Pin1 and p53 might regulate the Notch1 cleavage by presenilin1. It is clear that within the thymus, p53 is essential for elimination of DNA-damaged cells via apoptosis, and it appears that the loss of apoptotic pathways is critical to tumor formation within this cell type. The *Pin1*$^{-/-}$*p53*$^{-/-}$ mice phenotypes indicated that the physiological network between Pin1 and p53 might facilitate the elimination of DNA-damaged cells, partially by controlling the NIC level. In order to block tumorigenesis, isomerase activity of the dephosphorylated Pin1 probably facilitates p53 activation.

The roles of Pin1 played in cancer appear controversial. In general Pin1 protects against cancer. We found that ablation of both *Pin1* and *p53* accelerates thymic hyperplasia, whereas the thymocytes in the *pin1*$^{-/-}$*p53*$^{-/-}$ do not infiltrate other organs (5). These results suggest that roles of Pin1 in cancer may vary with dependence on organs, tissues, ages and genetic backgrounds. We speculate that posttranslational modifications and levels of functional homologues of Pin1 affect the functions of Pin1. We generated *Pin-1*$^{-/-}$*p53*$^{-/-}$ mice. NIC levels significantly increased in the thymocytes obtained from 12-week-old *Pin1*$^{-/-}$ *p53*$^{-/-}$ mice, and the up-regulated NIC may cause hyperplasia in the thymocytes. Presenilin1 level concomitantly increased with the NIC level in the thymocytes of the *Pin1*$^{-/-}$*p53*$^{-/-}$ mice. We also discovered that Pin1 could bind with NIC and induce NIC degradation, and that both Pin1 and p53 were key regulators of thymocyte proliferation via Notch1 activation.

p53 deficiency increases the expression of presenilin1 transcripts. Presenilin-1 is a component of the γ-secretase complex that acts as a cleavage protease, releasing NIC from Notch-1 and amyloid-β (Aβ) from the APP. Actually, p53-deficient mice exhibited accumulation of NIC in the thymus and Aβ in the brain. We could not detect the increase in NIC in the Pin1-deficient mice because cleavage of NIC from Notch1 was not affected by Pin1 deficiency. Our examinations revealed that NIC was up-regulated in the thymocytes in the *p53*$^{-/-}$ and *Pin1*$^{-/-}$*p53*$^{-/-}$ mice, and that the *Pin1*$^{-/-}$*p53*$^{-/-}$ mice exhibited NIC levels higher than those of *p53*$^{-/-}$ mice. p53 deficiency induced presenilin1 expression and thereby increased NIC production. Therefore, we speculated that Pin1 deficiency might lead to NIC stabilization, which is facilitated by the cleaving activity of presenilin1.

NIC might be one of the target substrates for Pinl because NIC associates with and is down-regulated by Pinl, and this down-regulation is inhibited by the addition of the proteasome inhibitor MG13. It has been shown that NIC is degraded via the ubiquitin-proteasome pathway. Pinl might isomerize phosphorylated NIC and render it susceptible to degradation via the ubiquitin-proteasome pathway. The Pinl-p53-NIC system might be essential for preventing the pathogenesis and progression of AD and cancer.

Alzheimer's Disease

AD is characterized by the presence of massive senile plaques (SPs) and NFTs, the 2 hallmark pathological features of AD, in the brain [6]. Both lesions result from the abnormal deposition of proteins that are normally distributed throughout the brain. SPs are extracellular deposits of the fibrillar amyloid β-peptide (Aβ), which is a cleavage product of the membrane-associated APP. NFTs are intracellular aggregated bundles of the microtubule-associated protein tau. Although the mechanism for AD pathogenesis remains elusive, accumulating data have indicated that tau and APP/Aβ are among the most important factors in the events leading to AD onset and progression. Notably, hyper-phosphorylation of the phosphoprotein tau is linked to its aggregation into filamentous structures, and the phosphorylation of APP at its cytoplasmic Thr-668 is linked to Aβ generation [7,8]. These observations suggest that the phosphorylation events are critical for understanding AD pathogenesis. Pinl has received increasing attention as a new regulator of the abovementioned phosphorylated proteins. Pinl is a partner molecule that performs phosphorylation-dependent binding on tau and APP. Among the known binding ligands for tau or APP, Pinl is uniquely situated such that it can bind both tau and APP.

The involvement of Pinl in AD was first described in association with tau. Pinl is predominantly a nuclear and mitotic regulator [6]. It binds and regulates the function of a subset of mitotic phosphoproteins, most of which are recognized by the monoclonal antibody MPM-2. MPM-2 also recognizes tau, which is phosphorylated at a number of Ser/Thr-Pro sites during mitosis. These observations prompted Lu and colleagues to examine whether the phosphorylated tau interacts with Pinl, and they discovered that tau indeed binds to the WW domain of Pinl. The longest isoform of human tau possesses 17 Ser/Thr-Pro sites. Of these, Thr-231 is required for interacting with Pinl when the site is phosphorylated. Further, Thr-231 plays a critical regulatory role because its phosphorylation greatly diminishes the ability of tau to bind and stabilize microtubules in the cell. The Thr-231 residue is hyper-phosphorylated in AD, and the authors discovered that Pinl binds hyper-phosphorylated tau obtained from the brain of AD patients. Surprisingly, the functional effect of the interac-

tion between Pin1 and tau phosphorylated at Thr-231 was restoration of the biological activity of phosphorylated tau. The tau functions regarding binding and stabilization of microtubules are regulated by its phosphorylation; tau binds the microtubules on dephosphorylation and does not bind the microtubules on phosphorylation. In the presence of Pin1, the impaired activity of hyperphosphorylated tau regarding promotion of the microtubule assembly was restored. The conformation of the Ser/Thr-Pro bonds is crucial for the action of the phosphatases. PP2A was able to dephosphorylate Ser/Thr-Pro only when it was in the *trans* conformation and not in the *cis* conformation. Thus, the restoring effect appears to depend on the prolyl isomerase activity of Pin1. Indeed, the *in vitro* experiments demonstrated that Pin1 binds phosphorylated Thr-231 and promotes dephosphorylation of tau via PP2A. The levels of soluble Pin1 are decreased in the brains of AD patients. This decrease might impair dephosphorylation of tau and restoration of its biological activity and have harmful effects on the balanced phosphorylation state of tau, leading to tau hyperphosphorylation and abnormal tau pathology. This assumption was supported by the study that used Pin1 knockout mice that we generated [1,6]. Pin1 knockout in mice induces age-dependent abnormalities, including hyperphosphorylation of endogenous tau, tau filament-formation, and neuronal degeneration, similar to those observed in the brains of patients with AD and tauropathy. The deficits observed in the Pin1-null mice suggest that Pin1 might protect against tau phosphorylation and thus inhibit the formation of NFTs.

Pin1 was further implicated in AD with its association to APP. APP undergoes proteolytic cleavage during and after its transport via the secretory pathway to the cell surface. In the major secretory pathway, some APP molecules are cleaved within the Aβ sequence by β-secretase, releasing the large soluble ectodomain fragment. With regard to the alternative amyloidogenic pathway, processing of the APP fraction by β-secretase secretes a slightly shorter ectodomain fragment. The membrane-bound proteolytic fragments generated by the β-secretase C83 and β-secretase C99 are further converted by the β-secretase cleavage within the transmembrane domain to form p3 (a truncated form of Aβ) and Aβ, respectively. Both these peptides terminate at either residue 40 or 42 of the Aβ sequence. The Thr-668 residue (human APP695 isoform numbering) preceding the Pro residue in the cytoplasmic APP domain is a known *in vivo* phosphorylation site, and its phosphorylation at Thr-668 is believed to regulate APP metabolism; this facilitates Aβ production. The Thr-668-Pro motif might serve as a potential binding site for Pin1, and we presume that Pin-1 binds APP and influences its metabolism. In order to verify this hypothesis, we investigated the *in vitro* and *in vivo* effects of Pin1 on APP metabolism. Herein, we discovered that Pin1 binds the phosphorylated Thr-668-Pro of APP in the HEK293 cells after it is cleaved at the β-secretase site, and this promotes its turnover, resulting

in increased Aβ production. In the brain of the Pinl-deficient mice, Aβ production was decreased as compared to that in their control littermates, indicating that Pinl facilitates an increase in Aβ production. Subsequent to our study, Pastorino *et al.* (2006) (8) also studied the interaction of Pinl and phosphorylated Thr-668 of APP. Inconsistent with our study, the authors showed that Pinl binds to full-length APP at phosphorylated Thr-668, and the effect of Pinl on Aβ generation was not stimulatory but inhibitory. Further studies are required to resolve the discrepancy between these 2 studies. Pinl is likely to interact with APP via recognition of the phosphorylated Thr-668 in its cytoplasmic tail and to influence the APP metabolism.

We recently found that the opposite regulation of oligodendrocyte apoptosis by JNK3 and Pinl after spinal cord injury (9). Pinl implicates in apoptosis, so it is a key regulator for neuron death.

Infection and Immunity

Peptidyl prolyl *cis/trans* isomerases (PPIases) catalyze the *cis/trans* isomerization of the peptidyl prolyl (X-Pro) bonds, and this *cis/trans* rotation of the peptide bond affects the spatial arrangement of the backbone segments in the proteins. The 3 phylogenetically conserved PPIase families involved in the catalysis associated with protein folding are the cyclophilins, FK506-binding proteins (FKBPs), and parvulins. The PPIase activity of the cyclophilins is inhibited by the immunosuppressant drug cyclosporin A, whereas that of the FKBPs is inhibited by FK506 and rapamycin. The parvulins are not inhibited by these immunosuppressant drugs.

Shen *et al.* reported that Pinl might be associated with the pathogenesis and malignancy of allergenic inflammation in asthma via stabilization of AU-rich element (ARE) mRNAs, including various cytokine mRNAs, and cytokine secretion. Pinl controls the post-translational levels of some cytokines that possess the 3′ untranslated region of AREs via interaction with the nucleoprotein in the ARE-binding complex AUF1. In resting cells, phosphorylated Pinl interacted to stabilize AUF1 and thereby degraded GM-CSF mRNA. Dephosphorylated Pinl exhibits enzymatic activity in activated eosinophils and may isomerize AUF1 to release ARE-mRNAs, resulting in the accumulation of ARE-mRNAs. Pinl is a critical regulator of cytokine mRNA turnover, which in turn controls the survival of activated eosinophils in the lungs of asthmatics. Since AUF-1 controls the decay of many ARE-mRNAs, the Pinl-AUF1 interaction might be important in cytokine production for inflammatory reactions and immune responses.

Pinl is necessary to transfer the cytokine signal in to the nucleus (10). These findings suggest that Pinl is strongly involved in infection and immunity systems.

Drug Discovery

As mentioned above, the post-translational modifications of Pin1 influence on its isomerase activity, and associate it with certain human pathologies. The post-translational modified form of Pin1 controls the phosphorylated transcription factors and transcriptional regulators, which alter the levels of some transcripts required in cell proliferation, differentiation, and apoptosis. Many transcription factors and their regulators that are involved in a variety of physiological process such as reproduction, development, and cellular homeostasis have been discovered to be substrates for Pin1. The components of activating protein-1 (AP-1); steroid receptor coactivator 3(SRC3), p53, p73; and the intracellular form of Notch1 (NIC) are physiologically associated with Pin1.

These new findings suggest that Pin1 plays an important role in the pathogenesis and therapeutics of human diseases such as cancer, AD, and asthma.

Acknowledgment

We thank Takahashi K, Shin R and Uchida C for admitting to summarize the original manuscript (11).

References

1 Fujimori, F., Takahashi, K., Uchida, C., and Uchida T (1999) Mice lacking Pin1 develop normally, but are defective in entering cell cycle from G(0) arrest. *Bioche. Biophy. Res. Commun.*, **265**: 658-663.

2 Liou, Y.C., Ryo, A., Huang, H.K., Lu, P.J., Bronson, R., Fujimori, F., Uchida, T., Hunter, T., and Lu, K.P. (2002) Loss of Pin1 function in the mouse causes phenotypes resemble cyclin D1-null phenotypes. *Proc. Natl. Acad. Sci. USA* **99**, 1335-1340.

3 Zacchi, P., Gostissa, M., Uchida, T., Salvagno, C., Avolio, F., Volinia, S., Ronai, Z., Blandino, G., Schneider, C., and Del Sal, G. (2002) The prolyl isomerase Pin1 reveals a mechanism to control p53 functions after genotoxic insults. *Nature*, **419**: 853-857.

4 Zheng, H., You, H., Zhou, X.Z., Murray, S.A., Uchida, T., Wulf, G., Gu, L., Tang, X., Lu, K.P., and Xiao, Z.X. (2002) The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response. *Nature*, **419**: 849-853.

5 Takahashi K, Akiyama H, Shimazaki K, Uchida C, Akiyama-Okunuki H, Tomita M, Fukumoto M., and Uchida T. (2007) Ablation of a peptidyl prolyl isomerase Pin1 from p53-null mice accelerated thymic hyperplasia by increasing the level of the intracellular form of Notch1. *Oncogene.*, **26**: 3835-3845.

6 Liou YC, Sun A, Ryo A, Zhou XZ, Yu ZX, Huang HK, Uchida T, Bronson R, Bing G, Li X, Hunter T., and Lu KP. (2003) Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegenera-

tion. *Nature.*, **424** : 556-561.

7 Akiyama H, Shin RW, Uchida C, Kitamoto T., and Uchida T. (2005) Pin1 promotes production of Alzheimer's amyloid beta from beta-cleaved amyloid precursor protein. *Biochem. Biophys. Res. Commun.*, **336** : 521-529.

8 Pastorino L, Sun A, Lu PJ, Zhou XZ, Balastik M, Finn G, Wulf G, Lim J, Li SH, Li X, Xia W, Nicholson LK, and Lu KP. (2006) The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production. *Nature.*, **440** : 528-534.

9 Li M, Tep C, Yune Y, Zhou Z, Uchida T, Lu P., and Yoon SO. (2007) Opposite regulation of oligodendrocyte apoptosis by JNK3 and Pin1 after spinal cord injury. *J. Neurosci.*, **27**, 8395-404.

10 Lufei C, Koh TH, Uchida, T., and Cao, X. (2007) Pin1 is required for the Ser727 phosphorylation-dependent Stat3 activity, *Oncogene*, **26**, 7656-7664.

11 Takahashi K, Uchida C, Shin RW, Shimazaki K and Uchida T. (2008) Prolyl isomerase Pin1 : New findings on post-translational modifications and physiological substrates in cancer, Alzheimer's disease and asthma, *Cell and Mol Life Sci.*, **65**, 359-375.