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Peptidyl-prolyl cis–trans isomerase Pin1 in ageing, cancer and Alzheimer disease

Tae Ho Lee, Lucia Pastorino and Kun Ping Lu*

Phosphorylation of proteins on serine or threonine residues preceding proline is a key signalling mechanism in diverse physiological and pathological processes. Pin1 (peptidyl-prolyl cis–trans isomerase) is the only enzyme known that can isomerise specific Ser/Thr-Pro peptide bonds after phosphorylation and regulate their conformational changes with high efficiency. These Pin1-catalysed conformational changes can have profound effects on phosphorylation signalling by regulating a spectrum of target activities. Interestingly, Pin1 deregulation is implicated in a number of diseases, notably ageing and age-related diseases, including cancer and Alzheimer disease. Pin1 is overexpressed in most human cancers; it activates numerous oncogenes or growth enhancers and also inactivates a large number of tumour suppressors or growth inhibitors. By contrast, ablation of Pin1 prevents cancer, but eventually leads to premature ageing and neurodegeneration. Consistent with its neuroprotective role, Pin1 has been shown to be inactivated in neurons of patients with Alzheimer disease. Therefore, Pin1-mediated phosphorylation-dependent prolyl isomerisation represents a unique signalling mechanism that has a pivotal role in the development of human diseases, and might offer an attractive new diagnostic and therapeutic target.

Protein phosphorylation on serine or threonine residues preceding proline (Ser/Thr-Pro) is a central signalling mechanism that regulates many key cellular processes, and its deregulation contributes to diverse pathological conditions, notably ageing and age-related disease such as cancer and Alzheimer disease (AD) (Refs 1, 2, 3). For example, in cancer, numerous oncogenes and tumour suppressors, which are directly regulated by Pro-directed phosphorylation, have been shown to be abnormally expressed or genetically mutated. Moreover, Pro-directed phosphorylation regulates many downstream cell cycle events,

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such as coordination of centrosome and DNA duplication cycles, and their incoordination can result in chromosome instability (Refs 1, 2, 4). Similarly, an interesting feature of degenerative neurons in the brains of patients with AD is increased mitotic phosphorylation of certain proteins on Ser/Thr-Pro motifs (Refs 2, 5). In addition, most stress-activated kinases, such as Pro-directed kinases and mitotic Cdc2 (CDK1 or cyclin-dependent kinase 1), are shown to be activated in AD brains (Refs 6, 7). These results suggest that AD pathogenesis involves activation of certain Pro-directed phosphorylation events common to the cell cycle, suggesting a connection between cancer and AD. Although Pro-directed phosphorylation has long been proposed to induce conformational changes (Ref. 8), little was known about the nature, significance and regulation of such protein conformations until the discovery of the phosphorylation-specific prolyl isomerase Pin1 (Refs 2, 9).

Pin1 (protein interacting with never in mitosis A 1) was originally identified as one of three human proteins, Pin1–Pin3, that physically interact with the *Aspergillus* mitotic kinase NIMA and functionally suppress its ability to induce mitotic catastrophe in yeast (Ref. 10). Pin1 is a unique prolyl isomerase that regulates protein function after phosphorylation on certain serine or threonine residues preceding proline (pSer/Thr-Pro). pSer/Thr-Pro motifs exist in two distinct cis and trans conformations, whose conversion is markedly slowed on phosphorylation but is specifically catalysed by Pin1 (Refs 2, 9, 11). The striking difference between Pin1 and other isomerases is that Pin1 specifically binds and isomerises phosphorylated Ser/Thr-Pro motifs, which are regulated by Pro-directed kinases (Refs 2, 10). Recent nuclear magnetic resonance structural analyses have shown that Pin1 accelerates the cis–trans conversion rate by more than 1000-fold compared with the uncatalysed reaction, presumably by lowering the N-C′ torsion barrier, and thus revealed how Pin1 binds its substrates to regulate their conformation (Refs 9, 12). Such unique substrate specificity is conferred by its two-domain structure. The WW domain of Pin1 binds only to specific pSer/Thr-Pro motifs, which target the Pin1 catalytic domain close to its substrate, where the PPIase domain isomerises the motifs and induces conformational changes in proteins, thus forming a 'double-check' mechanism (Refs 2, 9, 11). These Pin1-induced conformational changes after phosphorylation can have profound effects on phosphorylation signalling by regulating a spectrum of target activities.

Functionally, Pin1 is important in many cellular processes, including the cell cycle, cell signalling, transcription and splicing, DNA-damage responses, germ cell development and neuronal survival (Ref. 2). Moreover, Pin1 often uses several mechanisms to modulate several targets at various steps of a given cellular process to synergise and drive the cell in one direction. Thus, Pin1-catalysed prolyl isomerisation regulation might function as a timing mechanism, which would allow the cell to turn the function of phosphoproteins on or off with high efficiency during dynamic cellular processes. Alternatively, it might help to convert a protein kinase cascade into switch-like outputs, which act as an important signalling mechanism. Pin1 deregulation has an important role in an increasing number of pathological conditions, notably premature ageing, cancer and AD (Refs 12, 13, 14, 15, 16, 17, 18, 19, 20). The opposite effects of Pin1 on cancer and AD are further supported by Pin1 genetic association studies (Refs 21, 22, 23, 24), as well as by epidemiological studies of cancer and AD (Refs 25, 26). Thus, this review will focus on the role of Pin1 in ageing, cancer and AD and discuss its translational applications for new disease diagnosis and therapeutic interventions.

Pin1 and ageing

Ageing is a highly complex process of physiological functions and can be defined as the progressive functional decline of cells and organisms (Ref. 27). The rapid expansion of the global elderly population over the next 25 years will bring enormous challenges that are unprecedented in human history. Recent studies on various model systems and on centenarians have provided exciting clues to the mechanisms of ageing. However, the study of ageing is one of the few remaining frontiers of medical research. It is still unknown why some people develop memory loss, crippling diseases and functional disability as they grow older, whereas others experience good health and normal physical and cognitive function well into advanced age. There is an urgent need to explain

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the fundamental mechanisms underlying regulation of the ageing process and how ageing can lead to disease. A growing number of studies have shown that Pin1 has an important role in the regulation of ageing. Pin1-deficient mice develop normally and appear similar to wild-type mice for more than half of their lifespan, but eventually they display widespread premature ageing phenotypes, including reduced body size, atrophy of the testis and retina (Ref. 28), motor coordination and behavioural defects, neuron degeneration and loss (Refs 12, 29), osteoporosis, lordokyphosis, skin atrophy, accelerated telomere loss (Ref. 20) and germ cell depletion (Refs 30, 31) (Fig. 1). However, it remains to be determined how Pin1 regulates the basic mechanisms of ageing and whether it can control lifespan.

Pin1 and telomere maintenance

Telomeres cap the ends of linear chromosomes and have many important functions, including prevention of degradation, fusion and recombination of chromosome ends and blocking the activation of DNA-damage checkpoints (Refs 32, 33). Telomeres in most human somatic cells shorten during each cell division as a result of very low or absent activity of telomerase, a unique reverse transcriptase that extends telomeric sequences. Telomere shortening to a critical length and uncapping of the telomere lead to replicative senescence or crisis (Refs 34, 35). Indeed, telomere shortening is related to numerous age-associated diseases, including cancer, AD, hypertension, atherosclerosis and heart failure (or cardiovascular disease). Moreover, telomerase knockout in mice eventually causes telomere loss, limits cell proliferation and triggers premature ageing after four or five consecutive generations (Refs 36, 37, 38). In addition, telomere-associated proteins have been shown to have a central role in maintaining telomere homeostasis in human cells (Ref. 39). A key regulator in maintaining telomeres at reasonable length is the telomeric DNA-binding protein TRF1. TRF1 has been proposed to act in cis to progressively inhibit telomere elongation by telomerase because increased numbers of TRF1 molecules bind to telomeres (Refs 39, 40, 41, 42). TRF1 is tightly regulated during the cell cycle and is an important signalling molecule in mitotic regulation (Refs 43, 44, 45, 46). It has been shown that the F-box protein Fbx4 targets TRF1 and promotes its ubiquitin-mediated degradation (Refs 47, 48).

TRF1 was also identified as Pin2 in a combined screen for mitotic regulators. A recent study demonstrates an essential role for Pin1 in the regulation of TRF1/Pin2 stability, telomere maintenance and ageing (Ref. 20). Pin1 binds to TRF1 in vitro and in vivo after it has been phosphorylated on the conserved Thr149-Pro motif, only in mitotic cells, not in interphase cells (Ref. 20). Furthermore, inhibition of endogenous Pin1 function by several approaches renders endogenous or expressed TRF1 fully resistant to protein degradation, increases TRF1 telomere binding and also causes gradual telomere shortening. However, inhibition of Pin1 fails to cause telomere shortening in cells where TRF1 is also knocked down, because telomere elongation is not suppressed by telomere-bound TRF1. Moreover, knockdown of TRF1 results in telomere elongation in mouse cells, and Pin1-deficient mice show elevated levels of TRF1, telomere loss and various premature ageing phenotypes (Ref. 20). Within one generation, telomere loss and premature ageing phenotypes in Pin1-knockout mice (Ref. 20) are similar to those in telomerase-knockout mice after four or five generations (Refs 36, 37, 38). These results demonstrate the essential and conserved role of Pin1 in the regulation of TRF1 protein stability and maintenance of telomeres, deregulation of which contributes to premature ageing. Given the well-known role of Pin1 in growth signalling, Pin1-catalysed prolyl isomerisation might help in coordinating cell growth and telomere maintenance.

Pin1 and oxidative stress

Oxidative stresses can occur when a balance between the formation and removal of reactive oxygen species (ROS) is disturbed. ROS are implicated in age-related diseases such as cancer and neurodegeneration (Refs 49, 50). A recent study shows that Pin1 negatively regulates expression of the cyclin-dependent kinase inhibitor p27 (CDKN1B) through binding to phosphorylated FOXO4, which controls mitochondrial and oxidative stress, thus contributing to the maintenance of proper cellular functions (Ref. 51). FOXO4 is a member of the Forkhead boxO (FOXO) family. FOXO transcription factors have important roles in the suppression of ageing and tumours (Ref. 52).

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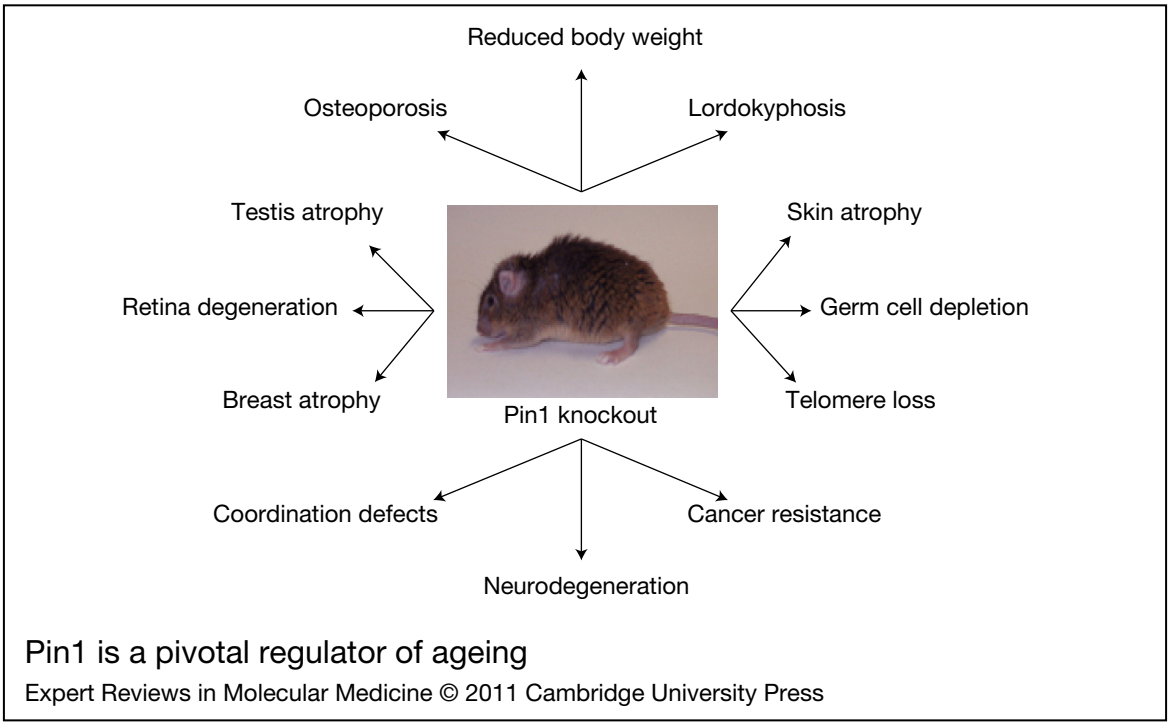

Figure 1. Pin1 is a pivotal regulator of ageing. Pin1-deficient mice develop normally and have normal phenotype for an extended period of time, but eventually show widespread signs of premature ageing, including overall appearance, reduced body size, testis and retina atrophy, motor coordination and behavioural defects, neuron degeneration and loss, osteoporosis, lordokyphosis, skin atrophy, accelerated telomere loss and germ cell depletion. A representative Pin1-knockout mouse is shown. Image adapted with permission from Nature Publishing Group (Ref. 29).

The FOXO orthologues DAF-16 and dFOXO also promote longevity in *Caenorhabditis elegans* and *Drosophila* (Ref. 52). Oxidative stress can induce binding of Pin1 and phosphorylated FOXO4 and inhibit its transcriptional activity by preventing its nuclear accumulation (Ref. 51). One of the functional consequences of the association of Pin1 and FOXO4 is to negatively regulate expression of p27 (Ref. 51). In addition, oxidative damage can induce binding to Pin1 and the 66 kDa isoform of the growth factor adaptor Shc ($p66^{Shc}$/SHC1; Src homology 2 domain-containing transforming protein 1) and mitochondrial import of $p66^{Shc}$ (Ref. 53). $p66^{Shc}$ is implicated in generation of ROS within mitochondria and translation of oxidative signals into cell death, indicating that it is involved in regulation of lifespan (Ref. 54). It is phosphorylated by protein kinase Cβ (PKCβ) in oxidative conditions and accumulates in mitochondria after it is recognised by Pin1, suggesting the involvement of Pin1 in oxidative stress and ageing (Ref. 53). Because FOXO4 is phosphorylated on several Ser/Thr-Pro sites and PKCβ is not a Pro-directed kinase, the Pin1 targeting sites in FOXO4 and $p66^{Shc}$ remain to be determined.

Pin1 is also modified by oxidation, which causes its inactivation in the early stages of AD, suggesting that Pin1 has an important role in the response to oxidative stress (Refs 2, 55, 56). A significant increase in Pin1 oxidation has been observed in the hippocampi of patients with mild cognitive impairment (MCI), and enzymatic activity of Pin1 is correspondingly decreased. Because MCI is an intermediate stage between normal cognitive ageing and early dementia or clinically probable AD, Pin1 is likely to be involved in the initial stages of development of AD, possibly through tangle formation and alteration of the cell cycle (Ref. 55). Indeed, Pin1 is subjected to oxidation in the hippocampus of AD patients, which appears to decrease its isomerase activity (Ref. 56). However, identification of the oxidation sites in Pin1 remains a major

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challenge. Recently, other members of the PPIase family – cyclophilins – have been shown to secrete a factor that is induced by oxidative stress, suggesting that phosphorylation-independent prolyl cis–trans isomerisation has an important role in regulating oxidative stress (Ref. 57).

Pin1 and the p53 family

The tumour suppressor p53 gene (*TP53*) family has an important role in maintaining genomic integrity, including cell cycle checkpoint and transcriptional activation (Refs 58, 59, 60). Loss of p53 function increases genomic instability and transformation, and *TP53* is mutated or deleted in more than 50% of human cancers (Ref. 61). Pin1 has been shown to regulate the function of the p53 family of proteins in response to DNA damage (Refs 62, 63, 64). Under normal conditions, p53 is targeted by MDM2 and degraded by the ubiquitin-mediated proteasome pathway. Pin1 can bind to DNA-damage-induced phosphorylation of p53 and prevent its binding to MDM2, which increases p53 protein stability (Refs 62, 63, 64). In addition, Pin1 is recruited to chromatin by p53 and promotes p53 binding and acetylation by p300 (Ref. 65). Pin1 is also required for dissociation of p53 from the relA-associated apoptosis inhibitor IASPP (PPP1R13L) (Ref. 65), suggesting that Pin1 regulates p53-dependent transactivation in several ways, by affecting protein stability, protein activity and protein–protein interaction. The tumour suppressor p73 (TP73) also belongs to the p53 gene family and shows strong structural and functional overlaps with p53 (Refs 66, 67). Pin1 is required for p73 protein stability because it promotes acetylation by p300 on DNA damage, and enhances the DNA-binding activity and transcriptional activity of p73 towards its target genes, including *TP21* (Ref. 68). Because Pin1 localises with p73 under normal conditions (Ref. 68), it remains to be determined how Pin1 regulates p73 binding and function in both normal and stressed conditions.

Pin1 and cancer

Pro-directed phosphorylation is a common and central signalling mechanism that controls cell proliferation and transformation, and its deregulation contributes to many human cancers. In fact, many oncogenic pathways are regulated by Pro-directed phosphorylation, which can initiate signalling cascades. Recent studies indicate that Pin1 regulates numerous Pro-directed phosphorylation signalling events and its overexpression or deregulation induces centrosome amplification, chromosome instability and cell transformation (Ref. 2).

Pin1 and its regulation in cancer

The connection between Pin1 and cancer was first suggested when Pin1 was found to be overexpressed in human cancer tissues (Refs 69, 70). These observations have been subsequently confirmed by demonstrations that Pin1 is significantly overexpressed in 38 out of 60 different human tumour types compared with expression in corresponding normal tissue (Ref. 71). Pin1 is overexpressed in most prevalently encountered cancers, including breast, prostate, lung and colon cancer (Ref. 71). Furthermore, increased Pin1 levels are positively correlated with a higher risk of, and a shorter period to, tumour recurrence following radical prostatectomy in a set of primary prostate cancers (Refs 72, 73). These results suggest that Pin1 overexpression is a specific event and a potential prognostic marker in human cancers. Consistent with its overexpression in cancers, with a few exceptions such as Myc and G1/S-specific cyclin E1 (CCNE1), Pin1 activates a number of oncogenes or growth enhancers and also inactivates many tumour suppressors or growth inhibitors (Table 1; Fig. 2). In addition to its overexpression in cancer, Pin1 is predominantly phosphorylated in normal tissues and cells, but becomes hypophosphorylated in cancer tissues and cells (Refs 69, 105), suggesting that Pin1 phosphorylation might have an important regulatory function in cancer. Indeed, death-associated protein kinase 1 (DAPK1), a known tumour suppressor, phosphorylates the Pin1 PPIase domain and inhibits its catalytic activity (Ref. 106). Moreover, DAPK1 inhibits the ability of Pin1 to induce centrosome amplification, chromosome instability and cell transformation (Ref. 106), illustrating an essential role of the catalytic activity for Pin1 function in cancer. Thus, Pin1 is a pivotal catalyst for tumourigenesis that acts by switching on or off numerous oncogenes or tumour suppressors, respectively, at several steps at the same time.

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Table 1. Pin1-interacting proteins that promote tumourigenesis

Substrate	Function	Binding site	Effect on Pin1	Activation (+) or inactivation (–)	Refs
AIB1/SRC3	Transactivator	–	Activity	+	74
Akt	Protein kinase	pThr92/450-Pro	Stability	+	75
Bax	Apoptosis	pThr167-Pro	Activity	–	76
Bcl-2	Antiapoptotic protein	–	Stability	–	77
Btk	Tyrosine kinase	pSer21/115-Pro	Stability	–	78
β-Catenin	Transcription factor	pSer246-Pro	Localisation, stability	+	70
C/EBP	Transcription factor	–	Activity	–	79
Cyclin D1	Transcription factor	pThr286-Pro	Localisation, stability	+	28
Daxx	Apoptosis	pSer178-Pro	Stability	–	80
FAK	Tyrosine kinase	pSer910-Pro	Activity	+	81
Fos	Transcription factor	–	Activity	+	82
FOXO4	Transcription factor	–	Localisation, activity	–	51
GRK2	G protein receptor	pSer670-Pro	Stability	–	83
Hbx	Transactivator	pSer41-Pro	Activity, stability	+	84
Jun	Transcription factor	pSer63/73-Pro	Activity, stability	+	69
Mcl-1	Apoptosis	pThr92/163-Pro	Stability	+	85, 86
Myb	Transactivator	pSer528-Pro	Activity	+	87
Neu	Growth factor receptor	–	Stability	+	88
NF-κB	Transcription factor	pThr254-Pro	Localisation, stability	+	89
Notch1	Growth factor	–	Activity	+	90
p70S6K	Ribosomal S6 kinases	–	Activity	+	91
p53	Transcription factor	–	Activity, stability	–[a]	62, 63, 64, 92
Plk1	Mitotic kinase	–	Binding activity	+	93, 94, 95
PML	Transcription factor	pSer403/505/518/527-Pro	Stability	–	96
Raf-1	Protein kinase	–	Activity	+	97
RARα	Transcriptional regulator	pSer77-Pro	Stability	–	98, 99
v-Rel	Transcription factor	pThr254-Pro	Localisation, stability	+	100
Smad	Transactivator	–	Stability	–	101
SMRT	Transcriptional corepressor	pSer1241/1469, Thr1445-Pro	Stability	–	102
Stat3	Transcription factor	pSer727-Pro	Activity	+	103
Tax	Viral oncoprotein	pSer160-Pro	Activity, stability	+	104
Pin2/TRF1	Telomere regulation	pThr149-Pro	Stability	–	20

[a]Based on the ability of Pin1 knockout to prevent cancers in *Tp53*-null mice.
Abbreviations: AIB1/SRC3, steroid receptor coactivator 3; Bax, Bcl-2-associated X protein; Btk, Bruton tyrosine kinase; C/EBP, CCAAT-enhancer-binding protein; Daxx, death-associated protein 6; FOXO4, Forkhead box protein; GRK2, G-protein-coupled receptor kinase 2; Hbx, hepatitis B virus X-protein; Mcl-1, myeloid cell leukaemia-1; NF-κB, nuclear factor-kappa B; p70S6K, ribosomal S6 kinases; Plk, polo-like kinase 1; PML, promyelocytic leukaemia protein; RARα, retinoic acid receptor alpha; SMRT, silencing mediator for retinoic acid and thyroid hormone receptor; STAT3, signal transducer and activator of transcription-3; Tax, T-cell leukaemia virus type 1; TRF1, telomeric repeat-binding factor 1.

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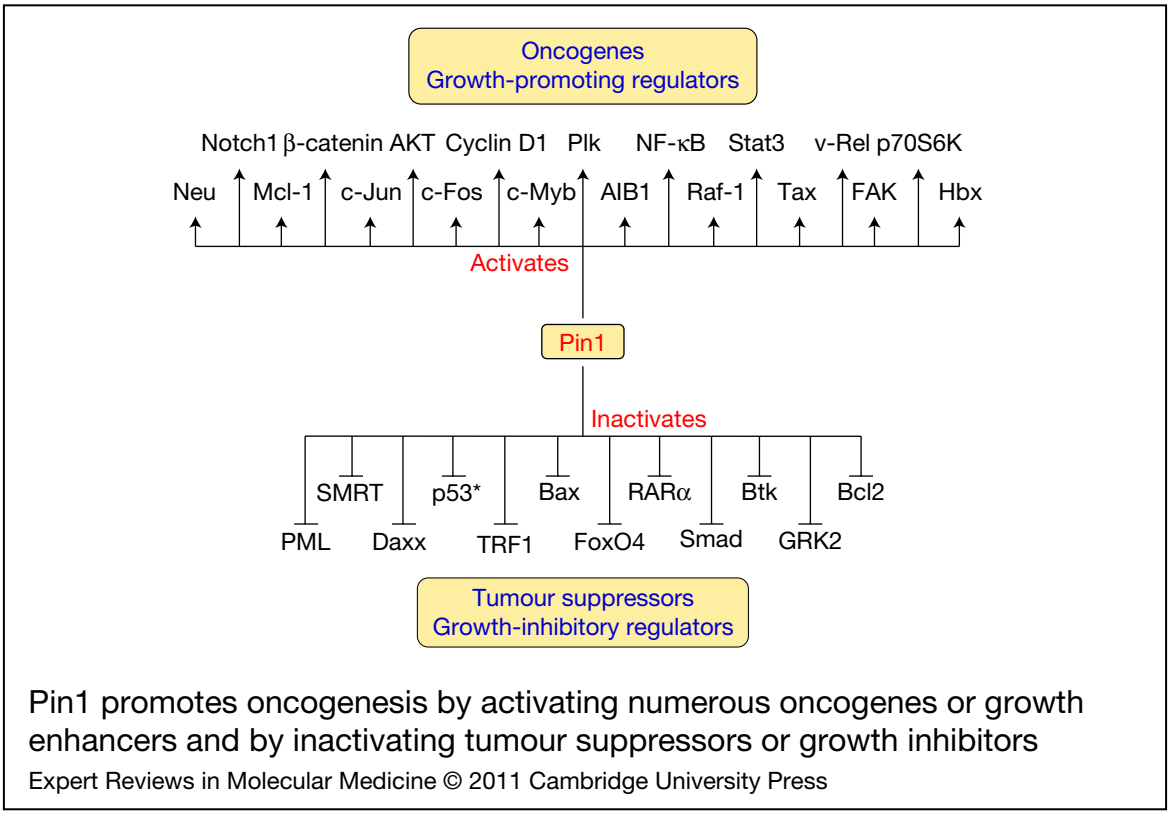

Figure 2. Pin1 promotes oncogenesis by activating numerous oncogenes or growth enhancers and by inactivating tumour suppressors or growth inhibitors. Pin1 promotes oncogenesis by activating numerous oncogenes or growth enhancers and by inactivating a large number of tumour suppressors or growth inhibitors. Pin1-catalysed cis-to-trans prolyl isomerisation regulates its protein substrate functions, such as catalytic activity levels, phosphorylation status, protein interaction, subcellular localisation and protein stability. Thus Pin1 amplifies oncogenic pathways by acting on several targets through the positive- and negative-feedback mechanisms. The asterisk indicates that the result is based on the ability of knockout of Pin1 to prevent cancers in *Tp53*-null mice.

Pin1 and oncogenic signalling pathways

Pin1 also increases cyclin D1 (CCND1) gene expression and protein stability by several mechanisms (Refs 28, 69, 70, 89). First, Pin1 interacts with the pSer63/73-Pro motifs in Jun by the activation of Jun N-terminal kinases (JNKs) in response to growth-stimulating conditions and positively regulates Jun transcriptional activity towards its target genes, which include cyclin D1 (Ref. 69). Second, Pin1 directly binds to β-catenin phosphorylated on the Ser246-Pro motif and regulates its turnover and subcellular localisation by interfering with its interaction with adenomatous polyposis coli protein, thereby enhancing transcriptional activity of β-catenin towards its target genes, which again include cyclin D1 (Ref. 70). Third, upon cytokine treatment, Pin1 can target the pThr254-Pro motif in NF-κB (NFKB1) and p65, and inhibits p65 binding to its inhibitor IκB, resulting in increased nuclear accumulation, protein stability and transcriptional activity of NF-κB towards its target genes, which include cyclin D1 and IκB (Ref. 89). Furthermore, Pin1 can directly bind to the pThr286-Pro motif of cyclin D1, which stabilises the cyclin D1 protein by preventing its nuclear export and ubiquitin-mediated degradation (Ref. 28). Moreover, deletion of *Pin1* in the mouse results in a phenotype that resembles that of the cyclin-D1-knockout mouse in some organs (Ref. 28). In addition, Pin1 is an E2F target gene, whose transcription is upregulated in response to activation of Her2/Neu or Ras (Ref. 28), suggesting that a positive-feedback loop exists (Fig. 3). Therefore, Her2/Neu or

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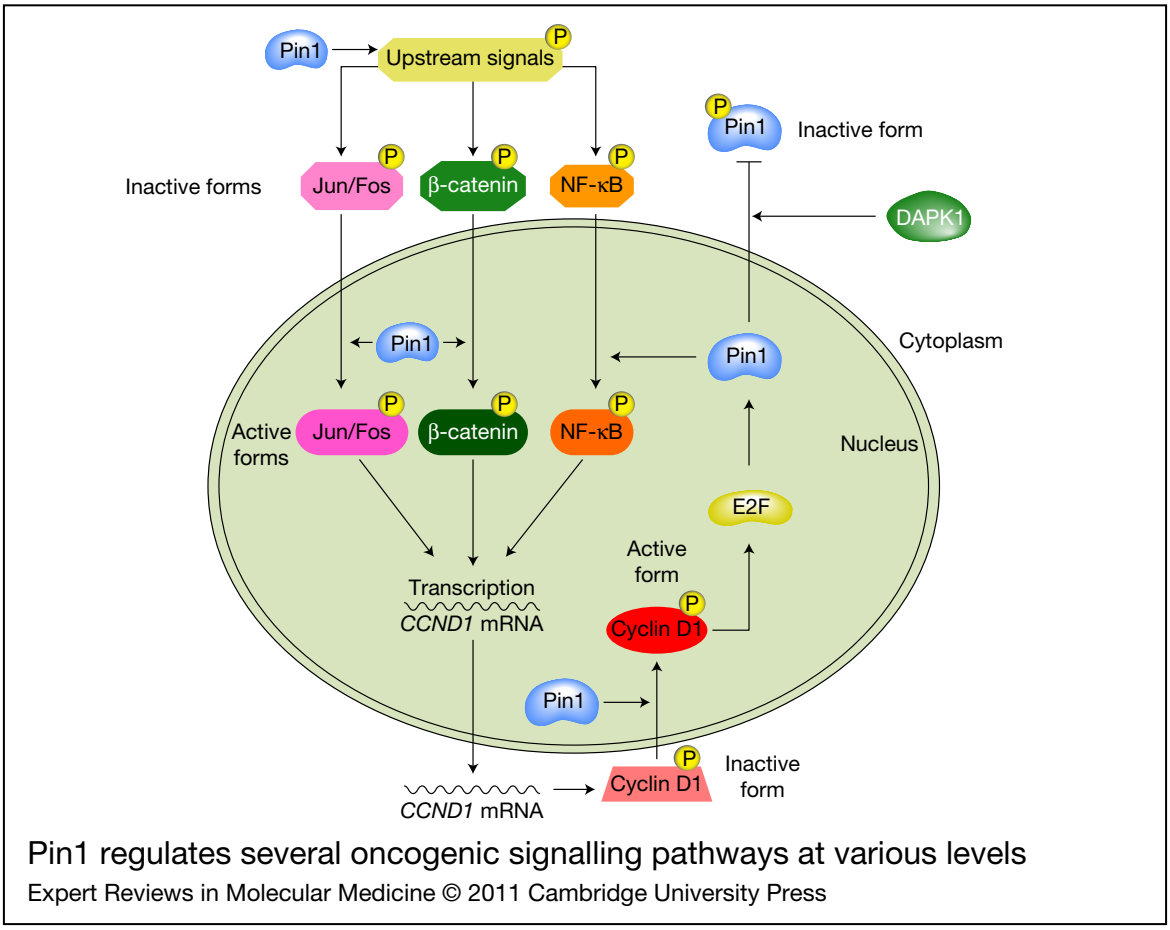

Figure 3. Pin1 regulates several oncogenic signalling pathways at various levels. Pin1 can increase transcription of cyclin D1 by modulating at least three different signalling pathways. Pin1 targets the pSer63/73-Pro motifs in Jun, multiple pSer/Thr-Pro motifs in Fos, the pThr246-Pro motif in β-catenin and the pThr254-Pro motif in the p65/RelA subunit of nuclear factor (NF)-κB to increase their nuclear translocation, protein stability and/or activity, thereby increasing expression of cyclin D1. Pin1 also directly binds to the pThr286-Pro motifs in cyclin D1 to increase its nuclear translocation and protein stability. Pin1 is an E2F target gene, whose transcription is upregulated in response to oncogenic activation, suggesting that a positive-feedback loop exists. In addition, the tumour suppressor death-associated protein kinase 1 (DAPK1) phosphorylates Pin1 and inhibits its catalytic activity.

Ras activation leads to an increase in Pin1 transcription by binding to the *Pin1* promoter through the E2F family of transcription factors, and Pin1, in turn, positively regulates cyclin D1 at the transcriptional and post-translational level.

Subsequent studies using cell culture and mouse models have demonstrated the crucial role of Pin1 in cancer development in vivo. Pin1 overexpression not only leads to transformed properties on normal mammary epithelial cells, but also can enhance the transformed phenotypes of Neu- or Ras-induced mammary epithelial cells (Ref. 28). By contrast, inhibition of Pin1 suppresses both cell proliferation and transformation induced by Neu- or Ras by cyclin D1 (Ref. 107). Pin1 can also inhibit a negative-feedback mechanism by promoting dephosphorylation of Raf, which activates Ras signalling (Ref. 97). Pin1 activates numerous other oncogenes or growth enhancers, including NF-κB (Ref. 89), Fos (Ref. 82), Stat3 (Ref. 103), Myb (Ref. 87), Notch1 (Ref. 90), AKT (Ref. 75), AIB1 (Ref. 74), Neu (Ref. 88), Mcl-1 (Ref. 85), Hbx (Ref. 84), Tax (Ref. 104), FAK (Ref. 81), p70S6K (Ref. 91) and v-Rel (Ref. 100), and also inactivates a large number of tumour suppressors or growth inhibitors, including

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FOXOs (Ref. 51), PML (Ref. 96), Daxx (Ref. 80), Bcl2 (Ref. 77), RARα (Ref. 98), SMRT (Ref. 102), Smad (Ref. 101), Bax (Ref. 76), Btk (Ref. 78), GRK2 (Ref. 83) and Pin2/TRF1 (Ref. 20) (Fig. 2). Finally, Pin1-knockout mice are almost completely resistant to cancer induced by overexpression of oncogenes, such as Neu or Ras (Ref. 107), or knockout of tumour suppressors, such as p53 (Ref. 92).

Pin1 and centrosome amplification

A recent study determined the role of Pin1 in centrosome duplication during the cell cycle (Ref. 18). The centrosome is a principal microtubule-organising centre in animal cells that facilitates the formation of mitotic spindle poles that segregate duplicated chromosomes between dividing cells (Refs 108, 109). Centrosome duplication is tightly regulated by the nuclear cell cycle and its deregulation can lead to abnormal centrosome numbers and aberrant mitosis, and eventual chromosome instability (Refs 4, 110). In fact, most tumours contain cells with centrosome abnormalities or amplification, which can indicate a poor clinical outcome (Refs 4, 110). Pin1 overexpression correlates with centrosome amplification in human cancer tissues (Ref. 18). Furthermore, Pin1 localises to, and copurifies with, centrosomes in interphase cells. Functionally, ablation or inhibition of Pin1 delays centrosome duplication during the S phase and suppresses centrosome amplification in S-phase-arrested cells. By contrast, Pin1 overexpression in S-phase-arrested cells induces centrosome amplification, aneuploidy and cell transformation. Moreover, mouse mammary tumour virus (MMTV)–Pin1 transgenic mice also develop centrosome amplification and eventually breast cancer (Ref. 18). Together with the findings that Pin1 expression is significantly increased at the G1–S transition and is also subjected to phosphorylation regulation during the cell cycle, these results indicate that Pin1 is crucial for coordinating centrosome duplication and DNA synthesis, and disruption of this process can induce centrosome amplification, chromosome instability and oncogenesis in vitro and in vivo.

Pin1 and the ER

Pin1 has also been shown to regulate both SRC-3 (the steroid receptor coactivator 3; also known as NCOA3) and SMRT (the corepressor silencing mediator for retinoic acid and thyroid hormone receptor; NCOR2) as downstream targets of Her2 to estrogen receptor (ER) signalling in breast cancer (Refs 74, 102, 111). SRC-3 is a transcriptional activation factor that interacts with steroid receptors in a ligand-dependent manner (Ref. 112). It is required for various physiological processes, such as cell proliferation, and is often overexpressed in breast and prostate cancers (Ref. 112). Pin1 interacts with phosphorylated SRC-3 and activates nuclear-receptor-regulated transcription of SRC-3 towards its steroid receptor target genes (Ref. 74). Pin1 also enhances interactions between SRC-3 and additional coregulator histone acetyltransferases such as CBP and p300 to recruit to the promoter-bound receptor coactivator complex (Ref. 74). Moreover, Pin1 promotes SRC-3 cellular turnover, suggesting that Pin1 functions as a secondary coactivator for steroid receptors by modulating conformational changes of SRC-3 protein and turnover of the activated SRC-3 oncoprotein. A recent study shows a role for Pin1 as a target for preventing improper ER activation by modulating SMRT protein stability (Refs 102, 111). SMRT is a coregulatory protein that mediates the repressive function of nuclear hormone receptors including ER (Ref. 113). Pin1 binds directly to phosphorylated SMRT and decreases SMRT protein stability, thereby affecting SMRT-dependent transcriptional repression (Ref. 102). Moreover, Pin1 is overexpressed in Her2-positive breast cancer and leads to the upregulation of erbB2 (receptor tyrosine protein kinase erbB-2, which is also known as Her2) (Ref. 88). Consequently, Pin1 is a central cofactor for coordinated Her2 activity and it is speculated that there is regulatory crosstalk between Pin1 overexpression and the constitutive activation of Her2-mediated transcriptional activation in breast cancer. These results suggest a potential therapeutic role for Pin1 in the treatment of endocrine-resistant breast cancers.

Pin1 and AD

AD is a progressive neurodegenerative disease associated with cognitive decline and characterised by the presence of both intracellular and extracellular lesions derived from distinct molecular pathways. Intracellular lesions are made up of hyperphosphorylated tau protein, which deposits in the form of aggregates called neurofibrillary tangles (NFTs)

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

(Ref. 114), whereas extracellular lesions are associated with the formation of insoluble deposits on the matrix of the brain, called β-amyloid plaques, which are derived from the pathological processing of the amyloid precursor protein APP (Ref. 115). The pathology of NFTs and plaques is very different, but despite this, both are needed to define AD (Ref. 116). Interestingly, although tau pathology is not specific to AD, because NFTs are also associated with a number of other neurodegenerative diseases (called tauopathies), a definite diagnosis of AD can be made only if NFTs are found in the presence of β-amyloid plaques. In this regard, the two pathologies could be linked through a common molecular regulator that can influence both NFT and plaque formation, through the regulation of both tau and APP. The prolyl isomerase Pin1 was identified as a major common regulator of these two pathologies in studies performed using Pin1-knockout cells and mice, as well as human AD samples. In particular, it was shown that Pin1 regulates the level of phosphorylated tau and NFT formation (Ref. 29) as well as the level of β-amyloid peptide (Aβ), which forms the core of the plaque (Ref. 12). Knockdown of Pin1 in mice results in age-dependent neurodegeneration of an AD type, with increased levels of phosphorylated tau and increased pathogenic processing of APP and insoluble Aβ. Pin1 is inhibited specifically in AD neurons by several different mechanisms (Ref. 29): levels of functional Pin1 were found to be reduced by oxidation (Refs 55, 56), Pin1 redistributed from the nucleus to the cytoplasm (Refs 117, 118), Pin1 colocalised with deposits of aggregated tau (Ref. 29), and reduced Pin1 expression correlated with increased vulnerability during neurodegeneration (Ref. 29). Interestingly, single-nucleotide polymorphisms (SNPs) have been found in the *PIN1* gene, which is located at 19p13.2, a locus that has been shown in some studies (Refs 21, 119), but not in others (Refs 120, 121), to relate to late-onset AD. Moreover, SNPs that prevent inhibition of Pin1 by brain-selective AP4 have been linked to a delayed onset of AD (Ref. 122). Because human *PIN1* is located at 19p13.2, a new locus for late-onset AD (Ref. 21), which is distinct from the *APOE4* locus 19p13.32 (Refs 123, 124), these observations support our hypothesis that Pin1 protects against AD.

Pin1 and tau pathology

The protein tau is critically involved in neurodegeneration. As a microtubule-binding protein, tau can regulate cytoskeleton structure by assembling microtubules; thus physiologically, tau serves as a controller of microtubule stability in the neuron. However, tau can become pathological when hyperphosphorylated (Ref. 125). Under these conditions, tau detaches from the microtubules, leading to destabilisation of the cytoskeletal structure and to cell death (Ref. 126). An additional pathological mechanism occurs when hyperphosphorylated tau precipitates into insoluble aggregates, forming paired helical filaments (PHFs), which comprise larger NFTs. This was also shown in animal models overexpressing the wild-type form of human tau (Ref. 127). When in the form of aggregates, tau is sequestered from the environment and cannot be dephosphorylated and thus cannot return to microtubule binding. In this respect, when it occurs, tau phosphorylation is a pathological event that cannot be reversed, and once triggered it causes neuronal death. It is indeed intriguing that phosphorylation controls the activity of tau to maintain cell structure, particularly because the kinases that phosphorylate tau, such as Cdk5, JNK and GSK3β (Refs 128, 129, 130, 131), are active during neurodegeneration (Ref. 132). In this regard, it could be speculated that when neurodegeneration starts, tau is phosphorylated, and this will cause irreversible modifications on tau that will lead to the death of neurons. It was previously suggested that such modifications would change the structure and conformation of tau (Refs 133, 134, 135, 136), affecting its capability to bind and to stabilise the microtubules.

Recently, it was shown that tau phosphorylation at Thr231 triggers conformational changes at this site that will initiate misfolding and will affect further changes in the conformation at different sites (Ref. 137). In this regard, among the many phosphorylatable sites present in tau, Thr231 seems to be key to determining the misfolding process and targeting this residue might be beneficial for prevention of wider tau misfolding and aggregation. Earlier studies have pointed to a possible role of Pin1 in tau pathology by regulation of phosphorylated Thr231. Pin1 can bind solely to Thr231 when phosphorylated and, in the presence of Pin1, hyperphosphorylated

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

tau is able to bind once again to microtubules in vitro (Ref. 117). This suggests that direct isomerisation at Thr231 is a post-phosphorylative mechanism to control the potential damage triggered by hyperphosphorylated tau, which is due to the activation of kinases during neurodegeneration. Moreover, a specific conformation of tau phosphorylated at Thr231 was found to colocalise with Pin1 in intracellular lesions. Using conformation-specific antibodies for phosphorylated tau at Thr231 in the brain of patients affected by AD, this form of tau was found to colocalise with Pin1 in intracellular granular aggregates (Ref. 138) that are different from the granulovacuolar lesions found in AD (Ref. 139). Similarly, Pin1 was also found to colocalise with tau aggregates in frontotemporal dementia (FTD) tauopathies (Ref. 140).

The presence of Pin1 in deposits containing tau suggests that Pin1 is somehow involved in their formation and that the cellular environment might be depleted of soluble Pin1. The consequence would be that less Pin1 is available to counteract the changes of conformation on newly phosphorylated tau at Thr231, and would probably sustain tauopathy. Interestingly, in AD samples, levels of Pin1 were decreased in brain preparations, which prevented the extraction of proteins from insoluble aggregates (Ref. 117), suggesting that the pool of soluble, and thus functional, Pin1 is decreased. These findings suggest that Pin1 protects against neurodegeneration caused by hyperphosphorylated tau.

This hypothesis was subsequently substantiated by demonstrations that selective gene ablation of *Pin1* in a mouse model expressing endogenous levels of native tau leads to an age-dependent neurodegenerative phenotype, characterised by hyperphosphorylation of tau at several sites and by the formation of PHFs (Ref. 29). Moreover, Pin1 expression inversely correlated with the levels of NFTs, because hippocampal neurons that showed increased levels of NFTs were characterised by lower levels of soluble, cytosolic Pin1, whereas those neurons with little or no NFTs had strong Pin1 expression. These findings suggest that levels of functional Pin1 inversely correlate with the vulnerability of neurons to develop lesions associated with hyperphosphorylated tau, and point to a possible role of Pin1 in the early stages of tauopathy. Conformation-specific hyperphosphorylated tau appears only in older Pin1-knockout animals, suggesting that the lack of Pin1 leads to accumulation of such forms of tau, which are possibly linked to the degenerative phenotype. Other studies have shown a role for Pin1 in promoting dephosphorylation of tau in vitro (Ref. 141).

The fact that selective gene ablation of *Pin1* could result in pathological modification on its substrate tau confirmed that direct isomerisation of specific substrates is key to the regulation of neurodegeneration and that specific isomers of tau are strictly related to the disease. Moreover, the fact that the ageing phenotype observed in the Pin1-knockout mice recapitulated that observed in human AD supports the idea that Pin1 has a crucial role in protecting against the disease; thus, increasing Pin1 levels in the brain could be a potential therapeutic approach to the treatment of AD.

A recent study showed that overexpression of Pin1 in the brain can revert the age-dependent tauopathy observed in Pin1-knockout mice (Ref. 142). As expected, overexpression of Pin1 in a transgenic animal model (with Pin1 expressed in postnatal neurons under the neuronal promoter Thy1) was protective from a phenotype linked to neurodegeneration. Pin1-transgenic mice showed a significant reduction in both stability of endogenous tau and phosphorylation of wild-type tau compared with levels in age-matched Pin1-knockout mice expressing tau at the same level. These data suggest that Pin1 reduces the availability of overall tau that can potentially be hyperphosphorylated in pathological conditions, and also regulates the equilibrium between physiological and pathological conformations of phosphorylated tau. Surprisingly, the ability of Pin1 to prevent a neurodegenerative phenotype associated with pathological tau was reverted when Pin1-knockout or Pin1-transgenic animals expressed the tau mutant P301L, which is associated with tauopathy observed in frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) (Refs 143, 144, 145). The presence of the P301L mutation stabilises tau levels, increases overall and conformation-specific tau phosphorylation in Pin1 transgenic mice, and causes peripheral neuropathy. Altogether, these data highlight the importance of direct isomerisation of proteins in

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

their native form as a fundamental factor in the initiation and progression of the molecular changes that lead to neurodegeneration in sporadic AD, and point directly to Pin1 as a key player in this process. Moreover, they open new perspectives on the use and construction of proper animal models for the study of the disease. The P301L tau mutant has been considered to be a good model for the study of AD-related tauopathy, because these animals develop massive tau hyperphosphorylation and formation of NFTs along with cognitive decline (Ref. 146). Given the crucial role of Pin1 in regulating the toxicity of endogenous tau, in the P301L mutant model, it cannot be excluded that the presence of the mutation maintains tau in a toxic configuration that cannot be reverted by Pin1, which can then be sustained despite the attempts of Pin1 to isomerise the protein. In this respect, one could speculate that the massive tauopathy observed in the mutant P301L tau model might not be representative of what occurs in the human brain in AD, especially considering that neither this nor other mutations on tau have been associated with this disease. Thus, the use of the Pin1-knockout mouse model should be a good tool for the study of age-dependent neurodegeneration.

Pin1 and β-amyloid pathology

β-Amyloid peptides accumulate as aggressive oligomers (Ref. 147) that impact synaptic transmission (Refs 148, 149) in AD. These oligomers further aggregate, forming the core of the β-amyloid plaque, which is the pathological hallmark of AD. The insoluble peptides have molecular weights of approximately 4 kDa and are generated from the precursor APP by pathological amyloidogenic processing, which involves sequential cleavage by the β-secretase BACE and γ-secretase on internalisation of the full-length protein from the plasma membrane (Ref. 150). The activity of BACE on APP generates a soluble stub called β-APP and an intracellular fragment called C99, which still contains the intact Aβ sequence. The activity of γ-secretase on C99 releases the intact Aβ (ranging in size from Aβ39 to Aβ43, with Aβ42 being the most aggressive) and a short fragment called the APP intracellular domain. APP can also be processed through a nonamyloidogenic pathway that involves the activity of α-secretase at the plasma membrane. This pathway generates α-APPs, which have neurotrophic properties (Ref. 151), it also prevents the production of Aβ and protects against AD (Ref. 152). Interestingly, the processing of APP can be regulated by phosphorylation at different sites (Ref. 153), and phosphorylation at the Thr668-Pro residue by Cdk5 (Refs 5, 154, 155) and GSK3β (Ref. 156) increases amyloid pathology in vivo and in vitro. Because the Thr668-Pro residue makes APP a suitable substrate for Pin1, it can be hypothesised that Pin1 binds to APP at this site and regulates the conformation of APP, influencing either the amyloidogenic or the nonamyloidogenic pathway (Ref. 12). Pin1 was found to bind specifically to phosphorylated Thr668 APP in vitro, which regulates the conformation of APP to favour nonamyloidogenic processing (Ref. 12). The Thr668-Pro motif in APP exists only in the trans conformation; however, the cis conformation forms after phosphorylation as a result of local hydrogen bond formation (Refs 157, 158). Pin1 greatly accelerates the cis to trans isomerisation of the phosphorylated Thr668-Pro motif of APP and also increases the nonamyloidogenic processing in vivo and in vitro (Ref. 12). These data suggest that phosphorylation at Thr668 changes the conformation of APP, making it toxic, and that Pin1 might protect from β-amyloid pathology by regulating the conformation of APP. These effects could depend on the capability of Pin1 to control the equilibrium between the cis and trans forms of the phosphorylated APP, and imply that the maintenance of such an equilibrium would protect against AD. Pin1-knockout animals were crossed with APPtg2576, an animal model characterised by age-dependent Aβ pathology and plaque deposition (Refs 159, 160), to study the processing of APP. The results showed an age-dependent shift in the processing of APP that would lead to an increase in the amyloidogenic versus nonamyloidogenic processing of APP. In particular, APP-Tg/Pin1-knockout mice at 6 months, but not at 2 months, had a massive increase in β-APPs and insoluble Aβ42 peptide paralleled with a decrease in α-APPs and the presence of aggregated Aβ42 in multivesicular bodies (Ref. 12), a feature indicating early AD pathology (Ref. 161). Similarly, the processing of APP and Aβ production seems to be affected when APP is expressed at endogenous levels in

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

Pin1-knockout mice, although at an older age, because Aβ42 peptide accumulated only in 15-month-old mice (Ref. 12). A role for Pin1 in modulating the processing of APP was confirmed in vitro by Pastorino and colleagues, who manipulated the expression of Pin1 in breast cancer cells and H4 human neuroglioma cells (Ref. 12). However contrasting results have been reported, where ablation of the *Pin1* gene protected against Aβ formation (Ref. 162). In this study, Akiyama and colleagues did not measure the generation of soluble and insoluble Aβ in the mouse brain during ageing, but only at an unspecified age. Second, they do not show binding of Pin1 to the full-length APP, but only to the C-terminal fragment C99. Given that (1) full-length APP is particularly phosphorylated at the Pin1-binding site Thr668 in AD (Refs 154, 155) and that (2) Aβ accumulates and forms insoluble aggregates as the animal ages, the study by Pastorino and co-workers (Ref. 12) gives a more comprehensive approach to understanding the role of Pin1 in modulating the processing of APP, ultimately leading to the accumulation of Aβ in the ageing Pin1-knockout mice.

It could then be speculated that Pin1 acts as an early factor in the development of Aβ pathology, because the absence of the *Pin1* gene causes increased pathological amyloidogenic processing of the precursor APP in APP transgenic mice at earlier stages than observed when expressing endogenous levels of Pin1, and before plaque formation. The fact that Pin1 might be involved in early stages of Aβ pathology is supported by Bulbarelli and colleagues (Ref. 163), who propose a compensatory upregulation of Pin1 as a consequence of toxic insult. In particular, they show that Pin1 activity is sustained and tau phosphorylation is decreased when cultured neurons are exposed to Aβ42 oligomers, which are early aggregates of Aβs.

Thus, Pin1 might protect from Aβ pathology in an age-dependent manner, possibly through regulation of the equilibrium between the cis and trans conformation of APP. In particular, in physiological conditions, Pin1 would favour the trans conformation of APP, increasing nonamyloidogenic processing. By contrast, in conditions associated with loss of Pin1 function or absence of Pin1, the cis form would accumulate, because isomerisation of the two forms would be lost, favouring amyloidogenic processing (Fig. 4).

This could be the case in a pathological scenario associated with AD: unlike physiological conditions characterised by low levels of phosphorylated APP at Thr668 and by physiological levels of Pin1, in AD pathology, levels of phosphorylated Thr668 are increased and Pin1 is possibly downregulated. Given the characteristics of Aβ pathology displayed by the Pin1-knockout model, it can be hypothesised that in AD, the equilibrium between the cis and trans conformation could shift towards the cis conformation, because of either an excessive level of APP phosphorylated at Thr668 or decreased levels of functional Pin1. More importantly, studies on Pin1, APP and tau (Refs 12, 29, 142) open up a new perspective on the study of protein isomerisation as a crucial mechanism to control post-phosphorylative events that might be detrimental to the life of the cell.

Pin1 in the nervous system and degenerating human brain

An increasing body of evidence supports the direct involvement of Pin1 in neurodegenerative diseases in the human brain. First, it was shown that levels of soluble Pin1 are decreased in the brains of AD patients compared with age-matched control brains (Ref. 117) and that Pin1 expression decreases in neurons that are vulnerable for tauopathy (Ref. 29). These data pointed to a possible protective role offered by Pin1 and suggested that a decrease in Pin1 levels might account for neurodegeneration. This hypothesis was further strengthened by the work of other laboratories, which showed that the reduction of either the expression (Ref. 164) or the activity of Pin1 (Refs 14, 55, 165) would associate with cognitive decline and AD. In particular, Wang and colleagues (Ref. 164) showed that levels of the Pin1 protein, but not its mRNA, were decreased in AD and in patients affected by MCI, a condition that often precedes early stages of AD, and that Pin1 activity inversely correlated with both tau phosphorylation and clinical cognitive scores in both AD and MCI patients. These data suggest that reduced Pin1 levels have possible consequences on both tauopathy and dementia, and were further supported by the findings of Butterfield and colleagues (Ref. 55), which demonstrate that Pin1 undergoes oxidation in the hippocampus of AD patients, paralleled by loss of function (Ref. 165).

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

Further evidence linked a possible reduction of the Pin1 protein level to the development of AD. Several SNPs were found to associate with AD and with decreased Pin1 levels in human cells (Ref. 22), although it is under debate whether these genetic mutations on the *PIN1* gene relate significantly to AD (Refs 120, 121, 166). However, the study of the genetics of *PIN1* could be useful to deepen our knowledge on protein function in the human brain. Recently, Ma and colleagues (Ref. 122) reported the existence of a polymorphism of Pin1 that reduces its own suppression because it inhibits binding to the brain-selective transcription factor AP-4, which would otherwise downregulate expression of Pin1. Because this polymorphism is associated with a delayed onset of sporadic AD in a Chinese population, this study opens new perspectives on mechanisms that could regulate and sustain Pin1 expression in AD and that could be potentially used for the treatment of this disease.

The presence of Pin1 in degenerating neurons has also been linked to amyotrophic lateral sclerosis (ALS) and to non-AD neurodegeneration associated with ageing, because Pin1 colocalises with hyperphosphorylated neurofilament protein in the spinal cord of ALS patients (Ref. 167) and with the aggregates of lipofuscin in elderly subjects (Ref. 168).

Pin1 mutant mice as models for human disease

Ageing

Pin1 is essential for cell division in some organisms such as budding yeast, *Aspergillus nidulans* and *Candida albicans* (Ref. 2). However, Pin1 ablation is dispensable for cell growth in others, including fission yeast. Initial analyses of young Pin1-knockout mice have shown that they are viable and develop normally with mild phenotypes (Ref. 169), suggesting that Pin1 is not required for cell division per se. However, further careful studies of adult Pin1-knockout mice have shown that they display a range of abnormalities similar to those of cyclin-D1-knockout mice (Ref. 28). The body weight of adult Pin1-knockout mice is significantly smaller at 71% of body weight in wild-type mice (Ref. 28). Moreover, Pin1-knockout mice showed retinal degeneration and Pin1-knockout pregnant females showed mammary gland proliferative impairment (Ref. 28). Pin1-knockout male mice display primordial germ cell depletion during embryonic development, along with testis atrophy in the adult (Refs 28, 30). Furthermore, Pin1-knockout mice display acceleration of telomere shortening and obvious premature ageing phenotypes within a single generation, including osteoporosis, lordokyphosis and skin atrophy (Ref. 20). In the brain, Pin1-knockout mice develop age-dependent neuropathy such as motor coordination and behavioural defects, as well as neuronal degeneration and loss (Refs 12, 29). These results suggest that Pin1 has a central role in regulating the ageing process in vivo.

Cancer

The critical roles of Pin1 in regulating cancer development have been studied in mouse models. Tissue-specific expression of Pin1 in mouse mammary gland induces centrosome amplification, eventually leading to mammary hyperplasia and malignant breast cancer with overamplified centrosomes (Ref. 18). Furthermore, Pin1 ablation in mice is highly effective in preventing oncogenic Neu or Ha-Ras, but not c-Myc, from inducing cyclin D1 and hence breast cancer (Ref. 107). Moreover, Pin1 ablation in p53-knockout mice completely prevents malignant tumour formation, although these mice exhibit accelerated thymic hyperplasia caused by a defective differentiation pathway (Ref. 92). In addition, mice expressing p53 with the deletion of Pin1-binding sites in the crucial Pro-rich domain of p53 do not affect the ability of p53 to inhibit tumour development induced by oncogenes (Ref. 170), demonstrating an essential role for Pin1 in oncogenic signalling pathways.

Alzheimer disease

The existence of a common regulator of both tau and APP pathology has great relevance to the search for an appropriate therapeutic target to be used in AD, possibly at early stages of the disease. Although it has emerged that Aβ pathology occurs before tau pathology in vivo (Refs 144, 146), it should be noted that these studies were carried out using animal models overexpressing pathogenic mutants of both APP and tau proteins: thus they are far from representing the real scenario of the brain of patients affected by sporadic AD. The presence at the same time of a pathogenic mutant at high amounts pushes the biological system towards

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

the exaggeration of toxic effects regulated by that protein. In particular, the presence of the mutation (tau P301L or APPswe) makes the protein very aggressive and capable of generating toxic species such as hyperphosphorylated tau and Aβs that can quickly aggregate into tangles and plaques, respectively, even at a young age. Given that AD develops late in life, these models do not fully represent the complex degenerative scenario associated with ageing that accompanies or causes AD in the human brain. However, it is important to point out that in AD, the levels of both tau and APP might be stabilised, for example, by an inefficient degradation system. The increased availability of the protein will certainly affect how much of the toxic species will be generated and this will influence progression of the disease. In this respect, animal models that are characterised by an accumulation or high levels of APP or tau are indeed useful for the study of AD.

Although Aβ seems to regulate tau hyperphosphorylation and aggregation, in vitro and in vivo (Refs 144, 146, 171), it remains under debate whether an effective treatment of AD pathology should first tackle the APP pathway and Aβ generation, or rather tau hyperphosphorylation and tangle formation. The studies performed using Pin1-knockout mice have identified in these animals a model that recapitulates some of the phenotypes found in AD, such as the age-dependent tangle and β-amyloid pathology that occur specifically from adulthood on in the life of the animal, similarly to the development of the disease in the human brain (Refs 12, 29, 142) (Fig. 5). Lack of Pin1 expression causes a degenerative phenotype related to tau and β-amyloid pathology when tau and APP are expressed at endogenous levels. In particular, it was observed that in the absence of Pin1 expression in the mouse brain, levels of endogenous tau are stabilised, with consequences on increased tau phosphorylation and PHF formation. Moreover, the Pin1-knockout mice also showed increased Aβ42 production. Thus, using the Pin1-knockout mouse model, it is possible to study the pathogenic phenotypes (i.e. tauopathy and AD) of proteins such as APP or tau expressed at endogenous levels, to allow a better understanding of the degenerative scenario associated with sporadic AD in the human brain.

Pin1 as a potential diagnostic and therapeutic target

Given its involvement in cancer and AD, Pin1 could be used in the diagnosis and therapy of these diseases. For example, Pin1 is inhibited by several mechanisms in AD neurons, including downregulation, oxidation and possible SNPs; an SNP that prevents Pin1 downregulation is associated with delayed onset of AD (Refs 21, 55, 56, 122, 164). Moreover, overexpression of Pin1 in postnatal neurons effectively prevents neurodegeneration in AD mouse models. These results suggest that analysis of Pin1 activity and promoter SNPs might provide some important information on vulnerability to neurodegeneration in AD. Possible approaches to treat neurodegeneration and AD could be aimed at preventing Pin1 downregulation by the brain-specific transcription factor AP4 or at increasing its function in neurons that are affected by the disease, for example by inhibiting Pin1 oxidation. However, further studies are needed to evaluate these interesting possibilities.

A number of unique properties make Pin1 a particularly attractive candidate for cancer diagnosis and treatment: (1) Human Pin1 is a readily purified, active enzyme that has high substrate specificity and well-defined active site structure (Ref. 11). (2) Pin1 uses its WW domain to target itself to the substrate, where its PPIase domain isomerises specific phosphorylated Ser/Thr-Pro motifs to regulate protein function (Ref. 2). Because the affinity of the PPIase domain towards substrates is quite weak, a potent catalytic inhibitor would effectively abolish Pin1 function. (3) Pin1 is overexpressed in ~60% of total human cancers, with high levels being correlated with poor clinical outcome (Refs 69, 70, 71, 72, 172, 173, 174, 175). By contrast, the Pin1 polymorphisms that reduce Pin1 expression are associated with reduced cancer risk in humans (Refs 23, 24). (4) Pin1 is required for activation or inactivation of numerous oncogenes or tumour suppressors, respectively (Refs 2, 20, 75, 88, 90, 96) (Fig. 2). (5) Pin1 overexpression transforms normal cells, enhances transformed phenotype by oncogenes and causes tumourigenesis in vitro and in vivo (Refs 18, 176). (6) Pin1 inhibition in cancer cells induces apoptosis or suppresses the transformed phenotype (Ref. 10). Pin1 phosphorylation by the tumour suppressor DAPK1 potently

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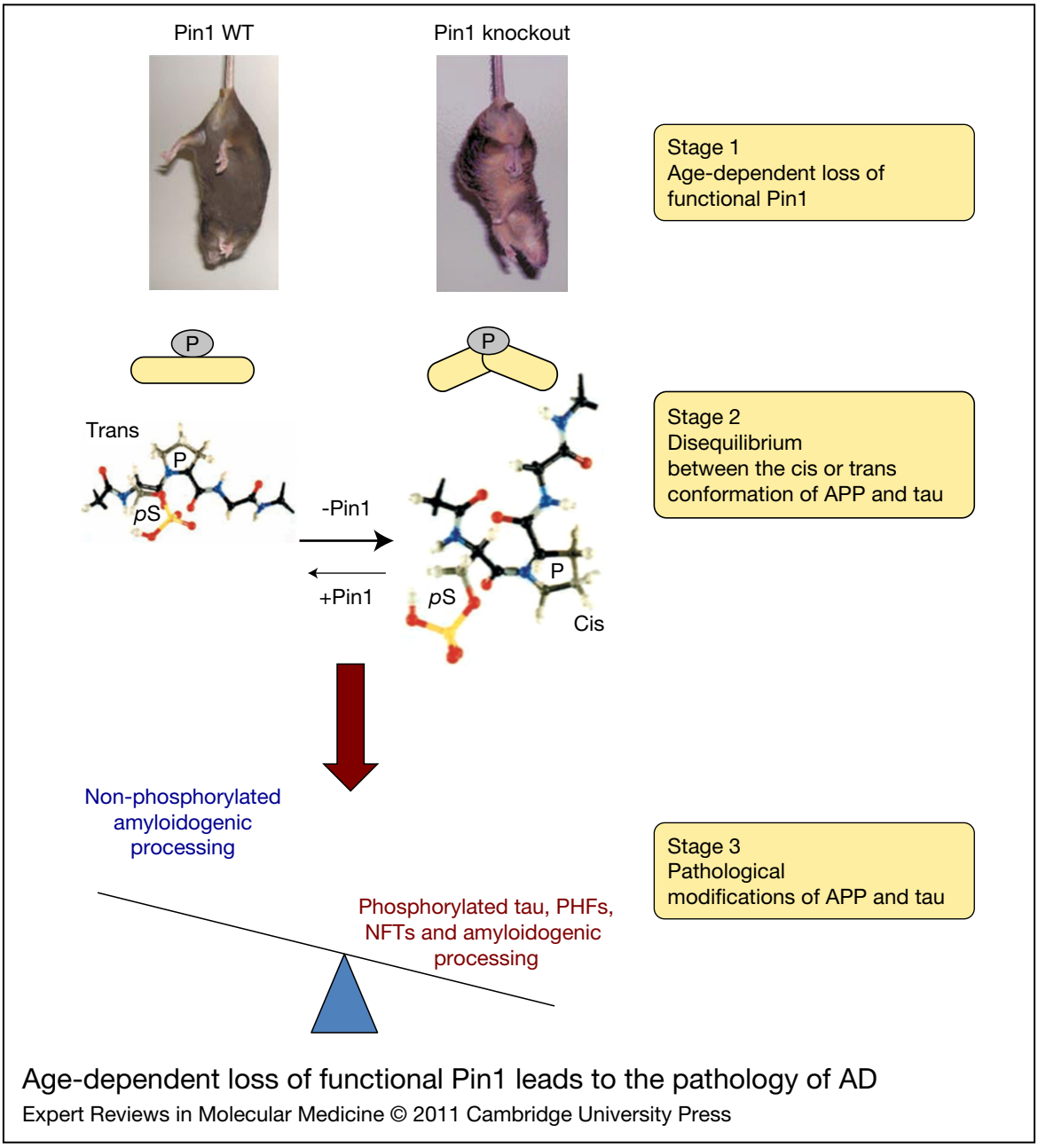

Figure 4. Age-dependent loss of functional Pin1 leads to the pathology of AD. During ageing, the levels of active Pin1 decrease as a result of changes in the synthesis or degradation of the protein or post-translational modifications, such as protein oxidation. In the Pin1-knockout animal model, the lack of expression of functionally active Pin1 leads to the development of a neuropathological phenotype associated with motor deficits and neurodegeneration. From a molecular point of view, the lower levels of active Pin1 are not sufficient to maintain the equilibrium between the cis and trans conformation of Pin1 substrates, in particular APP and tau. This results in the accumulation of specific conformations that are potentially toxic, causing the accumulation of hyperphosphorylated tau and NFTs, and increasing the amyloidogenic processing of APP, and Aβ formation. By modifying the conformation, and thus the molecular properties of both tau and APP, Pin1 controls the mechanism that switches their function from physiological to pathological, contributing to protection from the disease in different ways. Images adapted with permission from Nature Publishing Group (Ref. 29). Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer disease; APP, amyloid precursor protein; NFT, neurofibrillary tangle.

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

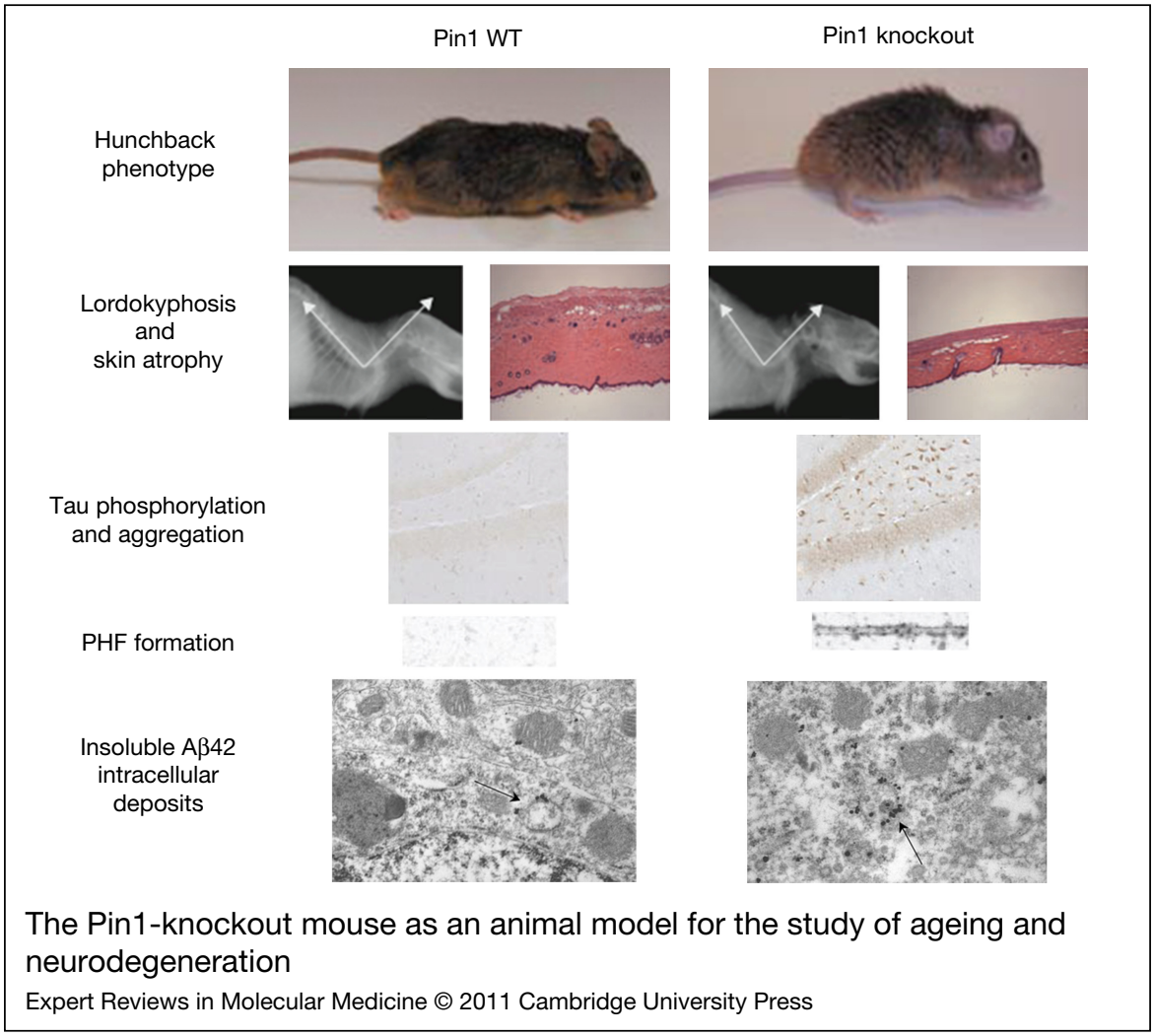

Figure 5. The Pin1-knockout mouse as an animal model for the study of ageing and neurodegeneration. Ablation of the Pin1 gene results in a phenotype that recapitulates the phenomena associated with ageing and neurodegeneration in AD, in the absence of transgenes such as mutant human APP or tau. This makes the Pin1-knockout mouse a unique animal model for the study of pathologies associated with ageing. Pin1-knockout mice are characterised by premature ageing due to changes in skeletal or muscular structure resulting in lordokyphosis and muscular atrophy. During ageing, these animals also develop massive tau phosphorylation and deposition in typical PHFs, as well as production and deposition of toxic Aβ species at an intracellular level. Pathological tau and Aβ lead to the neurodegenerative phenotype associated with lack of Pin1. Images adapted with permission from Nature Publishing Group (Refs 12, 20, 29). Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer disease; APP, amyloid precursor protein; PHF, paired helical filament.

suppresses Pin1 catalytic activity and cell function in inducing a transformed phenotype (Ref. 106). Moreover, Pin1 knockdown efficiently suppresses cancer cell growth in vitro and in mice (Ref. 177). (7) Pin1 knockout in mice prevents cancer development induced by overexpression of oncogenes such as Ras or Her2/Neu (Ref. 107) or knockout of tumour suppressors such as p53 (Ref. 92). Of note, Pin1-knockout mice develop age-dependent phenotypes, including retinal and neuronal degeneration, but only after more than half a lifespan without Pin1 (Refs 2, 20, 29). Together with the facts that Pin1-deficient mice develop

Accession information: doi:10.1017/S1462399411001906; Vol. 13; e21; June 2011

normally to adulthood and that Pin1 knockdown has no effect on normal cell growth (Ref. 177), these results suggest that short-term cancer treatments with Pin1 inhibitors might not have these age-related side effects, especially if the inhibitors cannot readily pass through the blood–brain barrier. Thus, Pin1 inhibitors might have the desired property to simultaneously inhibit several oncogenic pathways without general toxicity.

Although inhibitors of other PPIase families have been widely used clinically, they do not act on Pin1. Pin1 inhibitors identified include chemical compounds such as juglone and PiB, or peptide mimics identified by PPIase-based screens (Refs 178, 179, 180, 181, 182), binding-based screens, structure-based drug design (Refs 183, 184) or substrate-based drug design. However, they all lack the critically needed specificity, potency and cell permeability. For example, the natural product juglone is highly toxic because of its ability to covalently inactivate an active site cysteine in many proteins and enzymes, including the PPIases Pin1 (Ref. 178) and Par14 (Ref. 179). Similarly, PiB inhibits both Pin1 and Par14. The remaining Pin1 inhibitors cannot enter cells or simply do not have enough potency (Refs 180, 181, 182, 183, 184, 185, 186), with the possible exception of the less-potent Pin1 cyclic peptide inhibitor (Ref. 187). Highly specific and potent Pin1 inhibitors are urgently needed.

Conclusions

Pin1 is a crucial successful regulator of the signal transduction pathways that lead to ageing, cancer and AD. In particular, in neurodegeneration, Pin1 has been associated with the formation of the pathological hallmark lesions caused by hyperphosphorylation of tau and with Aβ toxicity, whereas in cancer Pin1 regulates the activity of numerous oncogenes and tumour suppressors involved in the control of cell proliferation and transformation. Through the literature discussed and through the animal models of Pin1-knockout and Pin1-transgenic mice, a higher activity of Pin1 has been associated with cancer, whereas a downregulation or lower expression of Pin1 is shown to lead to neuronal death.

The fact that Pin1 can control pathogenic mechanisms through the regulation of the conformation of protein substrates suggests that the maintenance of the equilibrium between cis and trans isomers of certain proteins is crucial to their physiological function. In pathological conditions, disruption of such equilibrium caused by alteration of the levels of functional Pin1 will cause loss of physiological function or gain of toxic function for Pin1 substrates, and would contribute to pathological conditions such as cancer and AD. Thus, prolyl isomerisation of proteins provides a fine mechanism that the cell uses to control the function of a protein, and Pin1 is a key enzyme that catalyses this reaction. In addition, the fact that changes in the activity of Pin1 are linked either to excessive cell proliferation or to cell death suggests that Pin1 might control the homeostasis of tissues through the control of the rate of cell growth. Interestingly, the development of neurological symptoms associated with AD inversely correlates with the development of cancer in elderly patients (Ref. 25). Together with the findings that levels of Pin1 are increased in certain types of cancer (Refs 107, 188) but are decreased in the brains of AD patients (Ref. 117), these observations suggest that changes in the levels of Pin1 could predispose individuals either to cancer or to neurodegeneration at a certain time of their life, particularly during ageing. Thus, the study of molecules that regulate the expression or the activity of Pin1 during ageing would certainly help to identify new pathways that act upstream of the isomerisation of a protein and would serve as tools to control the activity of Pin1 in age-related diseases. The identification of compounds that target Pin1 and its regulators might offer new therapies for age-related disease, especially cancer. Moreover, because there is no tool currently available to directly detect Pin1-catalysed cis–trans conformational changes in the cell, the development of such conformation-specific tools might provide new insights into the regulation of phosphorylation signalling under physiological and pathological conditions as well as offer new ideas for disease diagnosis and treatment.

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Further reading, resources and contacts

Publications

The following papers describe in detail the mechanisms of prolyl cis–trans isomerisation that are not discussed in this review. Other diseases involving Pin1 deregulation are also reviewed.

(continued on next page)

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Further reading, resources and contacts *(continued)*

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Websites

The UCSD-Nature Signaling Gateway Molecule Pages provide general information about Pin1, its regulation and interacting proteins:

http://www.signaling-gateway.org/molecule/

Features associated with this article

Figures

Figure 1. Pin1 is a pivotal regulator of ageing.
Figure 2. Pin1 promotes oncogenesis by activating numerous oncogenes or growth enhancers and by inactivating tumour suppressors or growth inhibitors.
Figure 3. Pin1 regulates several oncogenic signalling pathways at various levels.
Figure 4. Age-dependent loss of functional Pin1 leads to the pathology of AD.
Figure 5. The Pin1-knockout mouse as an animal model for the study of ageing and neurodegeneration.

Table

Table 1. Pin1-interacting proteins that promote tumourigenesis.

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