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REVIEW

Prolyl isomerase Pin1 in cancer

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Proline-directed phosphorylation is a posttranslational modification that is instrumental in regulating signaling from the plasma membrane to the nucleus, and its dysregulation contributes to cancer development. Protein interacting with never in mitosis A1 (Pin1), which is overexpressed in many types of cancer, isomerizes specific phosphorylated Ser/Thr-Pro bonds in many substrate proteins, including glycolytic enzyme, protein kinases, protein phosphatases, methyltransferase, lipid kinase, ubiquitin E3 ligase, DNA endonuclease, RNA polymerase, and transcription activators and regulators. This Pin1-mediated isomerization alters the structures and activities of these proteins, thereby regulating cell metabolism, cell mobility, cell cycle progression, cell proliferation, cell survival, apoptosis and tumor development.

***Keywords*:** Pin1; phosphorylation; cancer

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Introduction

Protein phosphorylation orchestrates the activation of signaling cascades in response to extra- and intracellular stimuli to control cell growth, proliferation and survival. These signaling events often include conformational changes in protein kinases and their substrates. Such conformational changes represent spatially and temporally controlled signaling mechanisms that regulate a spectrum of protein activities in physiological processes and diseases such as cancer [1, 2]. Conformational interconversions of the backbones of signaling proteins mediated by peptidyl prolyl *cis*-*trans* isomerization play a pivotal role in many aspects of cellular functions.

Proline-directed protein phosphorylation is a common and central signaling mechanism that has crucial roles in diverse cellular processes and controls cell proliferation and transformation, and its d**ys**regulation contributes to many human cancers [3]. Enzymes that are responsible for such phosphorylation belong to a large superfamily of proline-directed protein kinases, which include cyclin-dependent protein kinases (CDKs), mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases (ERKs), stress-activated protein kinases/c-Jun N-terminal kinases (SAPKs/JNKs), and p38 kinases, glycogen synthase kinase 3 (GSK3) and Polo-like kinases (PLKs) [4]. Proline exists in one of two distinct conformers, *cis* and *trans*. In peptides, proline interconversion occurs spontaneously and slowly in aqueous solution. Peptidyl prolyl *cis*/*trans* isomerases (PPIases) catalyze the *cis*-*trans* isomerization of peptidyl prolyl bonds, and this *cis*-*trans* rotation of the peptide bond affects the spatial arrangement of the backbone segments in the proteins. The three phylogenetically conserved PPIase families are cyclophilins, FK506-binding proteins and parvulins [5]. Protein interacting with never in mitosis A1 (Pin1), which is a member of the parvulin subfamily of PPIases and was originally identified in 1996 [6], specifically recognizes phosphorylated serine (S) or threonine (T) residues in pSer/Thr-Pro peptide sequences [6-9]. When a proline is positioned at the +1 position adjacent to a phosphorylated serine or threonine, the *cis*-*trans* conversion of the pSer/Thr-Pro bond is significantly more thermodynamically hindered than that of the Ser/Thr-Pro bond and rendered inaccessible to conventional PPIases, such as cyclophilins and FK506-binding proteins, but the pSer/Thr-Pro bond can be specifically recognized by Pin1 [5].

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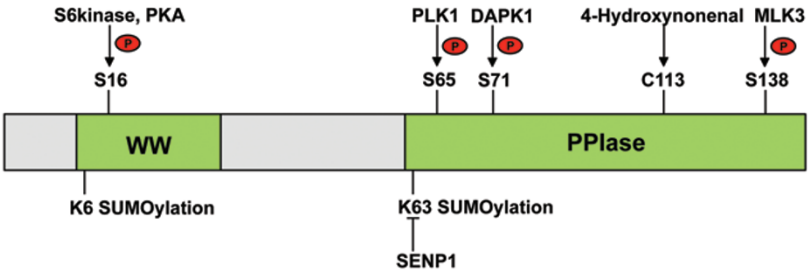

Figure 1 Schematic of the structure of Pin1 showing the regulatory posttranslational modification sites.

Pin1 has a modular domain architecture consisting of an N-terminal WW domain, a flexible linker and a C-terminal parvulin-type catalytic PPIase domain (Figure 1). The WW domain is responsible for Pin1's binding to pSer/Thr-Pro motifs in substrate proteins [5]. In addition, the Pin1 WW domain binds to the *cis* or *trans*, or both conformations of a pSer/Thr-Pro motif in a sequence-dependent manner [10]. For instance, the Pin1 WW domain exhibits almost the same affinity for the *cis* and *trans* conformations of the pThr668-Pro peptide of amyloid precursor protein and thus Pin1 catalyzes the interconversion between both conformations [11].

Pin1-catalyzed *cis*-*trans* isomerization could define subsequent enzyme actions on the targets. For example, Pin1-dependent isomerization promotes the dephosphorylation of *trans*-phospho-Ser/Thr-Pro motifs in its substrate proteins Cdc25C (pThr48-Pro and pThr67-Pro motifs) and Tau (pThr231-Pro motif) by the serine/threonine protein phosphatase 2A (PP2A) [12], while it promotes the dephosphorylation of the *cis*-phospho-Ser5-Pro motif of RNA polymerase II C-terminal domain (CTD) by the protein phosphatase Ssu72 [13]. Structural analyses of protein phosphatase Ssu72 in complex with the phosphorylated RNA polymerase II CTD substrate reveal that the CTD peptide is bound to the active site of Ssu72 with the phospho-Ser5-Pro peptide bond in the *cis* configuration [13, 14]. Pin1 isomerase activity greatly stimulates Ssu72 activity by increasing the pool of the CTD peptide in the *cis* configuration, which triggers the recruitment of Ssu72 and other transcription regulators to regulate general transcription. In contrast, small CTD phosphatase 1 (Scp1) binds to the *trans*-phospho-Ser5-Pro motif of CTD, which serves as its dephosphorylation substrate, and Pin1 has limited effect on Scp1 activity. Scp1 and the REST complex are recruited to CTD with the *trans*-phospho-Ser5-Pro motif to inhibit neuronal gene expression [15]. These findings underscore the importance of Pin1's function in regulating the recruitment of isomer-specific phosphatases for dephosphorylation of the substrate proteins.

Pin1 can also regulate the stability of substrate proteins by increasing or decreasing their ubiquitylation. Pin1 works with different E3 ubiquitin ligases to regulate degradation of proteins. Examples include the Skp1-Cullin-F-box (SCF)-type E3 ligase complexes for the degradation of c-Myc [16, 17] and cyclin E [18]; the suppressor of cytokine signaling (SOCS)1 (SOCS1 serves as the substrate-specific subunit of a Cullin 5 CRL E3 ligase complex, which is composed of Cul5, EloB/C and Rbx1) for the degradation of p65/RelA [19]; and HDM2 for the degradation of Che-1, an RNA polymerase II-binding protein that plays an important role in gene expression and the maintenance of the G2/M checkpoint [20].

Pin1 mediates conformational changes in its substrate proteins. However, some of Pin1's functions could be mediated simply by binding of its WW domain to pS/T-P motifs (i.e., a scaffolding activity) without involving its catalytic activities. To determine whether Pin1 acts on a pS/T-P target protein in a catalytic activity-dependent manner, Nakamura *et al*. [21] devised a method for generating isomer-specific antibodies for detecting *cis* and *trans* pS/T-P bonds in a sequence-dependent manner. Such antibodies can be used to directly assess the *cis*-*trans* isomerization state of individual pS/T-P bonds *in vivo* and provide evidence for their conformation-specific function or regulation [21].

Pin1 is regulated by extra- and intracellular stimuli, and governs the structures and functions of a broad range of signaling molecules, thus playing a pivotal role in tumor cell growth, survival, migration, invasion and metastasis**.** Mouse models have been used to investigate the critical roles of Pin1 in regulating tumor development. Transgenic overexpression of Pin1 in mouse mammary glands induces centrosome duplication, chromosome missegregation and aneuploidy, and results in mammary hyperplasia and malignant mammary tumors [22]. In addition, Pin1 ablation in mice is highly effective in preventing oncogenic Her2 or Ha-Ras from inducing cyclin

D1 expression and mouse mammary gland carcinoma [23]. Furthermore, Pin1 ablation in p53-knockout mice inhibits p53 deficiency-induced formation of lymphomas [24]. These findings support an instrumental role of Pin1 in promoting tumor development. This review will discuss the mechanisms underlying Pin1 regulation and the effect of Pin1's regulation of its substrates in tumor development.

Pin1 expression in human cancer

Pin1 overexpression is prevalent in human cancers. Analyses of 60 different human tumor types revealed that 38 of these tumors, including prostate, breast, lung, ovary and cervical tumors, and melanoma, have Pin1 overexpression in more than 10% of cases, as compared with the corresponding normal control tissues. Consistent with these findings, Pin1 expression in several types of human cancer is frequently found to be higher than that in their normal counterparts [25, 26]. Moreover, Pin1 expression has been linked to cancer prognosis. Two studies of Pin1 expression levels in 78 and 580 prostate tumor specimens, respectively, revealed that Pin1 overexpression positively correlates with a higher probability of and a shorter time to tumor recurrence following radical prostatectomy [27, 28]. High Pin1 expression has also been reported to correlate with poor survival and lymph node metastasis in non-small cell lung cancer patients and with disease progression in oral squamous cell carcinoma patients [29-31]. Similarly, Pin1 overexpression in esophageal squamous cell carcinoma correlated with lymph node metastasis and poor prognosis, and was found to be an independent prognostic factor for the disease [30]. In addition, Pin1 expression has been found to correlate with other tumor markers in humans. For example, Pin1 expression is associated with β-catenin accumulation in oral squamous cell carcinoma and closely correlates with cyclin D1 levels in esophageal squamous cell carcinoma and oral squamous cell carcinoma [30, 32, 33]. These findings support an important role for Pin1 in tumorigenesis and tumor progression.

Pin1 regulation in cancer

Pin1 can be regulated both transcriptionally and post-translationally. However, whether *PIN1* is amplified in cancer has not been intensively studied. *PIN1* is a direct target of E2 transcription factor 1 (E2F1), and Her2- and H-Ras-oncogenic signaling upregulates Pin1 expression via E2F1 [34]. In tamoxifen-resistant breast cancer cells, the phosphatidylinositol 3-kinase (PI3K) and p38 MAPK-dependent activation of E2F1 is critical for Pin1 induction [35]. *PIN1* promoter activity is decreased by a single-nucleotide polymorphism, −842G>C (rs2233678); this polymorphism has been associated with a decreased risk of squamous cell carcinoma of the head and neck [36, 37]. A different *PIN1* promoter single-nucleotide polymorphism, rs2287839, which is located within the consensus binding motif for the brain-selective transcriptional repressor AP4, almost completely abolishes the ability of AP4 to bind to the *PIN1* promoter and to suppress the *PIN1* promoter transcriptional activity [38].

Pin1 activity can also be regulated post-translationally (Figure 1). Death-associated protein kinase 1 (DAPK1) phosphorylates Pin1 at S71 in the PPIase catalytic active site to inactivate Pin1's catalytic activity and inhibit Pin1's nuclear localization and cellular function [39]. In contrast, mixed-lineage kinase 3 (MLK3), a MAPK kinase kinase (MAP3K) family member, phosphorylates Pin1 at S138 in the PPIase domain to increase its catalytic activity and nuclear translocation [40]. In addition, PLK1-dependent phosphorylation of Pin1 at S65 in the PPIase domain, which prevents the ubiquitylation and proteasome-dependent degradation of Pin1 without affecting its isomerase activity, stabilizes Pin1 [41]. The binding of Pin1 to its substrates can also be regulated by Pin1 phosphorylation. For instance, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment results in an interaction between the 90-kDa ribosomal protein S6 kinase 2 and Pin1, leading to the phosphorylation of Pin1 at S16 in the WW domain; this phosphorylation inhibits pSer/Thr-Pro target binding, thus regulating phosphoserine/threonine-binding activity of the WW domain and Pin1 function [42, 43]. In addition, upon forskolin treatment, activated PKA phosphorylates Pin1 at S16 in the WW domain, which results in the removal of Pin1 from nuclear speckles and redistribution of Pin1 throughout the cell. Expression of a mutant Pin1 carrying the WW domain S16A mutation induces mitotic block and apoptosis, and increases multinucleated cells [42], indicating that Pin1 phosphorylation at S16 has an essential role in cell cycle progression.

Oxidative stress induces lipid electrophiles to target Pin1 for modification. 4-hydroxynonenal, a common product of oxidative damage to membrane lipids, disrupts Pin1 activity by forming an adduct with Pin1 at C113, a residue in the active site that is essential for catalytic activity. Pin1 depletion partially protects breast cancer cells from 4-hydroxynonenal-induced toxicity [44].

SUMOylation is another regulatory modification for Pin1. SUMOylation of Pin1 on K6 in the WW domain and on K63 in the PPIase domain inhibits Pin1's activity and oncogenic function. SUMOylated Pin1 can be deSUMOylated by SUMO protease 1 (SENP1), which increas-

es Pin1 protein stability. Overexpression of SENP1 or disruption of Pin1 SUMOylation by mutations promotes Pin1's ability to induce centrosome amplification and cell transformation [45].

Pin1 regulates glycolytic enzymes for the Warburg effect and cell cycle progression

Pin1 plays a pivotal role in regulating the nuclear translocation of pyruvate kinase M2 (PKM2) and in subsequent regulation of the Warburg effect and cell cycle progression. The Warburg effect (also known as aerobic glycolysis) is characterized by elevated rates of glucose uptake and lactate production in tumor cells in the presence of oxygen; it enables tumor cells to function like fetal cells and use a large fraction of glucose metabolites to synthesize amino acids, phospholipids and nucleic acids that support tumor cell growth. PKM2, which catalyzes the final rate-limiting step of glycolysis by transferring a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP) to produce pyruvate and adenosine triphosphate (ATP), is instrumental in regulation of the Warburg effect [46, 47].

In response to activation of the epidermal growth factor (EGF) receptor (EGFR) and platelet-derived growth factor (PDGF) receptor, PKM2 translocates into the nucleus [48]. This translocation is mediated by the binding of ERK1/2 to PKM2 and phosphorylation of PKM2 at S37, which creates a binding motif for the Pin1 WW domain. The Pin1-dependent *cis-trans* isomerization of PKM2 dissociates the tetrameric PKM2 into monomers and exposes the inter- and/or intramolecularly masked nuclear localization sequence (NLS) encoded by *PKM* exon 10, leading to recognition of the NLS by importin-α and subsequent PKM2 nuclear translocation [46, 48, 49].

In the nucleus, PKM2 interacts with β-catenin phosphorylated by c-Src at Y333, an interaction that is required for their coordinate binding to the promoter regions of *CCND1* (encoding cyclin D1) and *MYC*, where PKM2 phosphorylates histone H3 at T11 [48, 50, 51]. This phosphorylation disrupts the binding of histone deacetylase 3 to histone H3, which leads to histone H3K9 acetylation and the subsequent transcriptional activation of *CCND1* and *MYC* [51, 52]. The increased expression of cyclin D1 promotes G1-S progression, whereas the enhanced expression of c-Myc upregulates expression of glycolysis-related proteins, including glucose transporter 1 (GLUT1) and lactate dehydrogenase A (LDHA), and polypyrimidine tract-binding protein (PTB) that facilitates the splicing of *PKM* pre-mRNA into *PKM2* mRNA, thereby upregulating PKM2 expression [50]. The increased expression of these glycolysis-related proteins promotes glucose uptake and lactate production. Thus, the Pin1-dependent nuclear translocation of PKM2 and the subsequent nuclear PKM2-regulated expression of the c-Myc and cyclin D1 promote the Warburg effect and cell cycle progression [46] (Table 1).

Pin1 regulates protein kinases and phosphatases

Protein phosphorylation regulated by protein kinases and phosphatases is involved in every important cellular activity in eukaryotic cells [53]. Pin1 regulates the functions of protein kinases and phosphatases at the plasma membrane, cytosol and nucleus.

Pin1 regulates receptor protein tyrosine kinases, Notch and non-receptor protein tyrosine kinase and phospha-

Table 1 Selected Pin1 substrates

Protein	Substrate regulation by Pin1	Pin1-binding motif	Kinase phosphorylating Pin1 substrates	Cellular consequence of Pin1 activation	Ref.
Glycolytic enzyme					
PKM2	Promoting nuclear translocation	S37	ERK	Promoting the Warburg effect and cell cycle progression	[46]
Protein kinases and receptors					
Her2	Increased stability			Increased tumor cell growth and chemoresistance	[54]
Notch1	Protein cleavage for activation, increased stability	T2512		Increased gene transcription	[55, 56]

FAK	FAK Y397 dephosphorylation	S910	ERK	Increased tumor cell migration, invasion, and metastasis	[57-59]
Raf-1	Dephosphorylation	S29, S289, S296, S301 and S642	ERK	Raf-1 activation	[62]
JNK1	Activation by inhibiting dephosphorylation	T183		Increased apoptosis	[64]
AKT	Increased stability	T92, T450		Increased tumor growth	[65]
P70S6K	Increased activity			Increased cell transformation	[66]
GRK2	Degradation	S670	CDK2	Cell cycle progression, cell survival regulation	[67]
CDK10	Ubiquitylation and degradation	T133		Activation of Raf/ERK MAPK pathway	[74]
Protein phosphatases					
PTP-PEST	Dephosphorylation of FAK Y397	S571	ERK	Tumor cell migration, invasion, and metastasis	[60, 61]
CDC25	Activating CDC2/ cyclin B	T48, T67		Cell cycle progression	[69, 71]
RNA polymerase					
RNA polymerase II	Dephosphorylation by Ssu72	S5		Transcription progression	[14, 15]
Methyltransferase					
SUV39H1	Protein ubiquitylation, degradation	S391		Reduced H3K9me3 and enhanced gene expression	[75]
Lipid kinase					
PIP4Ks	Inhibited kinase activity	T322 and S326	p38	Increased ROS production	[76]
E3 ubiquitin ligase					
Fbw7	Protein ubiquitylation and degradation	T205		Increased cell proliferation and transformation	[77]
DNA endonuclease					
RBBP8	Protein ubiquitylation, degradation	S276 and T315	CDK2	Inhibited DNA end resection	[79]
Transcription factors and co-regulators					
Transcription regulators promoting tumorigenesis					
Cyclin D1	Increased stability	T286	GSK3β	Increased gene transcription	[81]
Cyclin E	Degradation	S384	CDK2	G1/S arrest	[18, 78]
β-catenin	Increased stability	S246		Increased gene expression	[82]
c-Jun	Increased stability	S63/73	JNK	Increased gene expression	[26, 83]
c-Fos	Hetero-dimerization with other transcription factors	T232, T325, T331, and S374	ERK	Increased gene expression	[84]
RelA	Increased stability	T254		Increased gene expression	[19]

c-Myc	Promoter binding, and subsequent c-Myc degradation	T58, S62	ERK, CDK, GSK3β	Gene expression	[16, 17, 87, 88]
c-Myb	Increased transactivation activity	S528		Increased gene expression	[90]
SF-1	Increased SF-1-Pitx1 interaction	S203		Increased gene expression	[91]
Transcription factors in regulation of stem cells					
STAT3	Increased association with p300	S727		Increased gene expression, increased epithelial-mesenchymal transition	[96]
Nanog	Increased stability	S52, S65, S71, and T287		Stem cell renewal, tumor formation	[95]
Oct4	Increased stability	S12		Stem cell proliferation	[94]
Nuclear receptor					
ERα	Increased dimerization, stability, and activity	S118, S294	CDK2, ERK	Increased gene expression	[98-101]
Transcription regulators in tumor suppression					
Smad2/3	Ubiquitylation and degradation	T179, S204, S208, and S213 of Smad3		Inhibition of gene expression	[106]
SMRT	Degradation	S1241 and S1469	CDK2	Increased gene expression	[107]
FOXO4	Deubiquitylation			Suppression of p27 expression	[108]
p27	Increased stability	T187	CDK2	Increased gene expression	[109, 110]
PML	Degradation	S403, S505, S518 and S527	ERK	Increased cell proliferation	[113, 114]
RUNX3	Ubiquitylation and degradation	T209, T212, T231 and S214		Suppression of RUNX3-regulated gene expression	[104]
pRb	Hyperphosphorylation	S608 and S612	CDK	Increased gene expression	[105, 116]
p53	Transcriptional activation	S33, S46, T76, T81, T86, and S315	HIPK2, MAPK and others	Increased gene expression and enhanced apoptosis	[117-122]
p73	p73 acetylation by p300, increased p73 stability	S412, T442, T482	p38	Increased gene transcription for apoptosis	[123]
Apoptotic proteins					
BAX	Suppressed cleavage and mitochondrial translocation of BAX	T167	ERK	Anti-apoptosis	[125]
Daxx	Ubiquitylation and degradation	S178		Anti-apoptosis	[126, 127]
Bcl-2	Phosphorylation	S70 and S87		Apoptosis regulation	[128]
Mcl-1	Increased stability	T92 and T163	ERK	Anti-apoptosis	[129]
Survivin	Interaction with pro-caspase-9	T34	CDC2	Anti-apoptosis	[130]

tase at the plasma membrane

Pin1, which is highly expressed in Her2-positive breast cancer, interacts with the protein complex that contains ubiquitylated Her2. Pin1 depletion, which accelerates Her2 degradation, significantly suppresses the growth of Her2-positive tumor cells and sensitizes these tumor cells to the mammalian target of rapamycin (mTOR) inhibitor rapamycin [54].

Notch receptor signaling requires ligand-induced proteolytic cleavage to release the intracellular domain of Notch, which acts as a transcriptional activator in the nucleus. Deregulated Notch1 signaling has been implicated in mammary tumorigenesis, including breast cancer development. Pin1 binds to phosphorylated Notch1 and potentiates Notch1 intramembrane cleavage by γ-secretase, which increases the release of the active intracellular domain and enhances the activity of Notch1 in regulation of gene transcription [55]. In addition, Pin1 binds to phosphorylated T2512/P2513 within the Cdc4-phosphodegron of Notch1 and Notch 4, which is a motif known to be recognized by SCF-Fbw7α upon phosphorylation of T2512. Pin1-regulated prolyl-isomerization of the Notch proteins results in dephosphorylation of Notch1 and Notch4 by the PP2A phosphatase, preventing Fbw7α interaction and subsequent polyubiquitylation and degradation of Notch1 and Notch4. The Pin1-mediated activation of Notch1 and Notch4 promotes the self-renewal and chemoresistance of breast cancer stem cells, as well as tumor growth and metastasis formation [56]. Intriguingly, the distal BS1 element on the *PIN1* promoter is directly regulated by Notch1 signaling; thus, these two factors form a feed-forward loop. The mutual regulation of these two proteins in cancer is supported by a strong correlation between Pin1 overexpression and high levels of activated Notch1 in human breast cancer specimens [55].

Focal adhesion kinase (FAK) is a focal adhesion-associated non-receptor protein-tyrosine kinase involved in cellular adhesion and spreading and cell migration. Activation of EGFR and Ras, which has been detected in many types of human cancer and found to promote tumor cell migration and metastasis, induces FAK Y397 dephosphorylation and FAK inhibition, a process mediated by the Ras downstream Cdc42-p21-activated kinase 1 (PAK1)-MAPK kinase (MAP2K) (MEK)-ERK signaling cascade [57-59]. ERK phosphorylates FAK at S910 and the protein-tyrosine phosphatase (PTP)-proline-, glutamate-, serine- and threonine-rich sequence (PEST) at S571, leading to the recruitment of Pin1 and PTP-PEST to colocalize with FAK at the lamellipodia of migrating cells. Pin1 binding and the prolyl isomerization of both FAK and PTP-PEST cause PTP-PEST to interact with and dephosphorylate FAK Y397. FAK inhibition mediated by Pin1-dependent, PTP-PEST-regulated FAK Y397 dephosphorylation promotes the disassembly of focal adhesions and the turnover of lamellipodia and enhances Ras-induced cell migration, invasion and metastasis [58-61].

Pin1 regulates cytosolic protein kinases in the MAPK and AKT pathways

The Raf-1 kinase, an important signaling molecule at the top of the ERK MAPK pathway, functions in the Ras pathway to transmit mitogenic, differentiative and oncogenic signals to the downstream kinases MEK and ERK. Activated ERK phosphorylates Raf-1 at S29, S289, S296, S301 and S642 to block the interaction between Ras and Raf-1, thereby inhibiting Raf-1 activity via a negative feedback mechanism. The hyperphosphorylated and desensitized Raf-1 subsequently undergoes *cis-trans* isomerization by Pin1, which leads to Raf-1's dephosphorylation by PP2A, and this dephosphorylation returns Raf-1 to a signaling-competent state. Deficiency or inhibition of Pin1 blocks PDGF-induced Raf-1 activation, indicating that Pin1 is required for Raf-1 to be recycled for activation after PDGF stimulation, as it is required for PP2A dephosphorylation of Raf-1 at the ERK phosphorylation sites [62]. In contrast, in response to EGF stimulation, Pin1 does not interact with Raf-1 but instead interacts with MEK1. This interaction enhances the EGF-induced phosphorylation of MEK1/2 and ERK1/2 and induces activator protein (AP)2-dependent Her-2 expression, which in turn promotes neoplastic cell transformation [63]. In contrast to ERK, which is indirectly regulated by Pin1 through Pin1 action on ERK-upstream protein kinases, JNK1 (another member of the MAPK family) is directly regulated by Pin1. In response to hydrogen peroxide stimulation, Pin1 binds to the phosphorylated T183 of JNK1 in the activation loop and catalyzes the isomerization of the phospho-T183-Pro motif from *trans* to *cis* conformation. This structural change stabilizes JNK1 activity by inhibiting PP2A-dependent JNK1 dephosphorylation, thus enhancing the interaction between JNK1 and its substrates, such as c-Jun and activating transcription factor (ATF)2, increasing the binding of T-cell factor (TCF) β1 to the interleukin-2 (IL-2) promoter to induce IL-2 production and enhancing JNK1-induced apoptosis [64].

In addition to the MAPK pathways, AKT-regulated signaling is another central regulatory pathway in many cellular functions. Pin1 regulates AKT stability and phosphorylation at S473. Pin1 binds to the phosphorylated Thr-Pro motifs at AKT T92 and T450 (the "turn motif"), and this binding is required for the maintenance of AKT

stability and AKT phosphorylation at S473. Pin1 deficiency or depletion by its shRNA compromises Pin1's ability to protect AKT from degradation. In addition, expression levels of Pin1 and levels of AKT phosphorylation at S473 have been found to be strongly correlated in multiple cancer types. In breast cancer patients, the combination of high AKT-pS473 levels with high Pin1 expression levels predicts poorer prognosis than either factor alone [65].

p70 ribosomal protein S6 kinase (p70S6K), which is downstream of AKT/mTOR signaling, also binds to Pin1 in a p70S6K phosphorylation-dependent manner. In hepatocarcinoma cells, this complex formation enhances the phosphorylation of p70S6K at T389, the activity of p70S6K and the interaction between p70S6K and ERK1/2 in response to insulin stimulation, thereby increasing ERK1/2 activity, AP1 transactivation activity and neoplastic cellular transformation [66]. These results suggest that Pin1 plays an important role in insulin-promoted hepatocarcinoma tumorigenesis.

Pin1 modulates protein kinase and phosphatase actions in cell cycle regulation

Cell cycle progression requires orchestrated changes in the activity or expression levels of a variety of key signaling proteins. G protein-coupled receptor kinase 2 (GRK2), which plays a central role in G protein-coupled receptor regulation, is phosphorylated by the cyclin-dependent kinase 2 (CDK2) at S670 near the C-terminus. This phosphorylation triggers GRK2's binding to Pin1 and its subsequent degradation during the G2/M transition. Prevention of GRK2 phosphorylation at S670 by mutation to Ala impedes normal GRK2 downregulation and markedly delays cell cycle progression in a receptor-independent fashion [67]. In addition, doxorubicin-induced activation of the G2/M checkpoint stabilizes GRK2 levels, which inversely correlate with the p53 response and the induction of apoptosis, suggesting that GRK2 participates in the regulatory network that controls cell cycle arrest and survival under such conditions [67].

The cyclin-dependent kinase CDC2 (the human homolog of CDK1)/cyclin B complex regulates mitosis entry and progression. CDC2 is negatively regulated by phosphorylations at its T14 and Y15 residues, which are mediated by the Myt1 and Wee1 kinases, respectively. These phosphorylations can be reversed by the CDC25 phosphatase. Pin1 interacts with Myt1 in a phosphorylation-dependent manner and potentially modulates Myt1-regulated mitosis entry [68]. In addition, Pin1 interacts with CDC25 and PLK1, a CDC25-regulatory serine/threonine-protein kinase that phosphorylates the amino-terminal domain of CDC25 [69, 70]. The interaction between Pin1 and phosphorylated CDC25 promotes a conformational change in CDC25 and increases its ability to activate and maintain CDC2/cyclin B activity [69, 71]. These results suggest that Pin1 modulates cell cycle control through its interaction with CDC25 and its regulation of CDC25-dependent CDC2/cyclin B activity.

Pin1 also regulates CDK10, which uses cyclin M as an activating cyclin. Cyclin M/CDK10 phosphorylates the transcription factor ETS2 and causes ETS2 degradation by the proteasome [72]. CDK10 depletion increases the activity of the transcription factor ETS2 to induce expression of the *RAF1* gene, resulting in ERK MAPK pathway activation [73]. Pin1 interacts with pT133 of CDK10, leading to the ubiquitylation and degradation of CDK10 [74]. Overexpression of CDK10 sensitizes tamoxifen-resistant breast cancer cells to 4-hydroxytamoxifen treatment [74].

Pin1 regulates suppressor of variegation 3-9 homolog 1 methyltransferase (SUV39H1)

One of the most abundant and stable histone modifications is the methylation of histone H3 on K9 (H3K9), which is involved in both gene repression and heterochromatin formation. Suppressor of variegation 3-9 homolog 1 (SUV39H1) is a major methyltransferase responsible for histone H3K9 trimethylation (H3K9me3). Pin1 interacts with the Ser391-Pro motif of SUV39H1 in a phosphorylation-dependent manner and promotes the ubiquitylation-mediated degradation of SUV39H1, which reduces H3K9me3 levels. Pin1 depletion elevates SUV39H1 expression, increases H3K9me3 levels and inhibits cancer cell tumorigenicity. In addition, SUV39H1 overexpression abrogates Pin1 overexpression-promoted tumor growth, and in human breast cancer specimens Pin1 levels have been found to be negatively correlated with SUV39H1 and H3K9me3 levels [75].

Pin1 regulates lipid kinase, ubiquitin E3 ligase and DNA endonuclease

Phosphatidylinositol-5-phosphate (PtdIns5P) 4-kinase (PIP4K) is a lipid-modifying enzyme that phosphorylates and thereby reduces the amount of PtdIns5P by converting it into phosphatidylinositol 4,5-bisphosphate. PIP4K-Pin1 interaction, which depends on the p38-mediated phosphorylation of PIP4K at T322 and S326, has been found to inhibit PIP4K activity *in vitro*. However, whether Pin1-mediated inhibition of PIP4K occurs in cells remains unknown. Compared with $Pin1^{+/+}$ mouse embryonic fibroblasts (MEFs), $Pin1^{-/-}$ MEFs had higher PtdIn-

s5P levels in response to hydrogen peroxide and higher expression of the genes required to eliminate reactive oxygen species (ROS), which increased the anti-oxidative ability and survival of these cells. Reducing the amount of PtdIns5P in *Pin1*$^{-/-}$ MEFs decreased the expression of these genes and increased the accumulation of cellular ROS, suggesting that Pin1 regulates PtdIns5P, a redox second messenger that alters gene expression to calibrate cells' sensitivity to ROS [76].

The F-box protein Fbw7, which has three alternatively spliced α, β and γ isoforms, and binds to phosphorylated targets, is the substrate recognition component of an SCF E3 ligase complex and a tumor suppressor that targets oncoproteins such as cyclin E. Pin1 interacts with Fbw7 phosphorylated at T205 and disrupts Fbw7 dimerization to promote Fbw7 self-ubiquitylation and degradation. Fbw7 abundance inversely correlates with Pin1 expression in human colon cancer specimens, and Pin1 overexpression reduces Fbw7 abundance and promotes cell proliferation and transformation [77]. In addition to regulating Fbw7 abundance and the subsequent Fbw7-dependent degradation of its substrate proteins, Pin1 regulates the interaction between Fbw7 and phosphorylated cyclin E, Notch1/4 and c-Myc by altering the local conformation of these oncoproteins, and thus modulating their polyubiquitylation and degradation [17, 56, 78]. These findings suggest that Pin1 could manipulate Fbw7 functions and in doing so contribute to oncogenesis through multiple mechanisms, including Pin1-mediated Fbw7 degradation, disruption of Fbw7-mediated oncoprotein degradation due to reduced Fbw7 levels and interruption of the association between Fbw7 and these oncoproteins.

DNA endonuclease retinoblastoma-binding protein 8 (RBBP8), also known as C-terminal binding protein interacting protein (CtIP), cooperates with the MRE11-RAD50-NBS1 complex in the processing of meiotic and mitotic double-strand breaks. Pin1 interacts with CtIP phosphorylated at S276 and T315 in a CDK2 activity-dependent manner. Pin1 isomerizes CtIP and promotes its ubiquitylation and degradation. CtIP phosphor-mutant expression promotes the hyper-resection of DNA double-strand breaks, supporting the notion that Pin1-dependent CtIP isomerization is a key regulatory mechanism that restricts the resection of DNA double-strand breaks in late S/G2 phase [79].

Pin1 regulates transcription factors

Pin1 regulates transcription factors and transcription regulators including cyclin D1, cyclin E and c-Myc in central pathways important for tumor development

Cyclin D1, which is important for cell cycle progression and cell proliferation, is a direct substrate of Pin1. Pin1 binds to cyclin D1 phosphorylated at Thr286-Pro by GSK3β and increases cyclin D1 levels in the nucleus and stabilizes cyclin D1. Pin1-deficient mice and cyclin D1-deficient mice share characteristics that include retinal hypoplasia and impaired mammary gland development. In pregnant Pin1-deficient mice, cyclin D1 levels are significantly reduced in many tissues, including the retina and breast epithelium, and these mice fail to undergo the massive proliferative changes in breast epithelium associated with pregnancy [80, 81].

Pin1 regulates cyclin D1 function not only at the post-translational level but also at the transcriptional level through transcription factors, such as β-catenin, c-Jun and p65/RelA nuclear factor-κB (NF-κB) subunit, which upregulate *CCND1* transcription [3, 19, 26, 82, 83]. Pin1 binds to the phosphorylated Ser246-Pro motif of β-catenin, which lies next to the adenomatous polyposis coli protein (APC)-binding site, thus inhibiting β-catenin's interaction with APC and increasing β-catenin's stability and its translocation into the nucleus. Pin1 increases the transcription of β-catenin target genes, thereby enhancing cyclin D1 and c-Myc expression. In addition, β-catenin expression levels are decreased in Pin1-deficient mice but increased and correlated with Pin1 overexpression in human breast cancer [82]. In response to the activation of JNK or oncogenic Ras, Pin1 binds to c-Jun phosphorylated at Ser63/73-Pro motifs in the transactivation domain by JNK and inhibits c-Jun ubiquitylation to increase its stability, thereby enhancing the transcriptional activity of c-Jun towards the *cyclin D1* promoter [26, 83]. In addition, ERK phosphorylates multiple residues, including T232, T325, T331 and S374, within the C-terminal transactivation domain of c-Fos, which can heterodimerize with c-Jun or other AP1 family members. Pin1 binds to phosphorylated c-Fos, enhancing c-Fos's transcriptional response to polypeptide growth factors that stimulate ERK [84]. Cyclin D1 can also be upregulated by NF-κB activation. In response to cytokine stimulation, Pin1 binds to the phospho-Thr254-Pro motif in p65/RelA and inhibits the binding of p65/RelA to the NF-κB inhibitor IκBα, which increases the nuclear accumulation and stability of p65/RelA by inhibiting SOCS1-dependent ubiquitylation of p65/RelA, leading to enhanced NF-κB activity [19]. Pin1 markedly enhances the transformation of primary lymphocytes by the human c-Rel protein and increases cell transformation by the potent viral oncoprotein v-Rel [85]. Thus, Pin1 regulates the expression levels of cyclin D1 and its regulators β-catenin, c-Jun and p65/RelA NF-κB, which also affect the expression of a broad spectrum of downstream genes to influence many important cellular activities.

Besides regulating cyclin D1 expression, Pin1 also regulates the stability of cyclin E. Pin1's binding to the cyclin E-CDK2 complex depends on CDK2-mediated phosphorylation of cyclin E at S384 [18], which promotes cyclin E degradation mediated by the sequential functions of SCF-Fbw7α and SCF-Fbw7γ. First, SCF-Fbw7α binds to a complex containing cyclin E, CDK2 and Pin1, and promotes the activity of Pin1 to isomerize a non-canonical proline-proline bond in cyclin E (the P381-P382 bond), but does not directly ubiquitylate cyclin E. Pin1-mediated cyclin E isomerization leads to the release of cyclin E from SCF-Fbw7α, which enables SCF-Fbw7γ to bind to and polyubiquitylate cyclin E [78]. Fbw7-driven cyclin E degradation is impaired in cells expressing oncogenic RasG12V. Oncogenic Ras activity both inhibits cyclin E-Fbw7 binding and cyclin E ubiquitylation, and potentiates cyclin E-induced genetic instability, which may serve as a mechanism through which Ras mutations promote tumorigenesis [86].

In addition to regulating c-Myc expression by enhancing β-catenin transactivation [82], Pin1 directly binds to c-Myc phosphorylated at S62 by ERK or CDK and promotes the recruitment of c-Myc and its coactivators, including histone acetyltransferases GCN5 and p300, to target gene promoters [87]. Subsequent removal of c-Myc from the promoters is mediated by GSK3β-dependent c-Myc phosphorylation at T58. This in turn leads to Pin1-mediated c-Myc isomerization, which facilitates c-Myc S62 dephosphorylation by PP2A and subsequent recognition of c-Myc phosphorylated at T58 by SCF-Fbw7 for proteasomal degradation [16, 17, 87, 88]. Thus, through the binding of Pin1 to c-Myc phosphorylated at either S62 or T58, Pin1 is involved in both the recruitment and dissociation of c-Myc to and from target gene promoters. The tumor suppressor scaffold protein Axin1 is required for the formation of the degradation complex for c-Myc, containing GSK3β, Pin1 and PP2A [89]. In some cancer cells in which the degradation of c-Myc is impaired due to Axin1 loss [87], c-Myc stabilization and Pin1-enhanced c-Myc DNA binding promote oncogenesis [87]. These findings show that in cancer cells Pin1-mediated activation of c-Myc could be uncoupled from its ability to promote c-Myc degradation.

Pin1 also regulates the c-Myb protein in a c-Myb S528 phosphorylation-dependent manner and enhances its transactivation activity [90]. In addition, Pin1 activates SF-1, Pitx1 and Egr-1 transcription factors, either by modulating their stability or by increasing their protein-protein interactions. For instance, Pin1 is required for the S203 phosphorylation-dependent ubiquitylation of SF-1, which facilitates the interaction between SF-1 and Pitx1, and therefore enhances SF-1 transcriptional activity [91]. Thus, Pin1 regulates a broad range of transcription factors in central pathways that are important for tumor development.

Pin1 regulates transcription factors involved in stem cell self-renewal and pluripotency

Pin1 knockout mice display some abnormalities owing to decreased cell proliferation, including decreased body weight, retinal degeneration, a neurodegenerative condition reminiscent of Alzheimer's disease and impaired mammary gland development [80, 92]. These mice also exhibit testicular atrophy with significantly impaired proliferation of primordial germ cells and the progressive loss of spermatogenic cells [93], suggesting that the loss of Pin1 function causes impaired maintenance and proliferation of germ-related stem cells.

Prominent characteristics of pluripotent stem cells are their unique capacity to self-renew and their pluripotency. The activation of signal transducer and activator of transcription 3 (STAT3), Nanog and Oct4 plays instrumental roles in these processes [94-96]. STAT3 belongs to a family of latent cytoplasmic transcription factors important for tumor progression. In response to cytokine or growth factor stimulation, Pin1 recognizes phospho-Ser727-Pro of STAT3, leading to the binding of transcriptional coactivator p300 to STAT3, and thereby promotes STAT3 transcriptional activity and target gene expression to enhance STAT3-mediated epithelial-mesenchymal transition of breast cancer cells [96].

Nanog, a transcription factor crucial for the self-renewal of embryonic stem cells, is phosphorylated at multiple Ser/Thr-Pro motifs (i.e., S52, S65, S71 and T287). This phosphorylation promotes the interaction between Nanog and Pin1. Pin1 stabilizes Nanog by suppressing its ubiquitylation. In immunodeficient mice, inhibition of Pin1 activity or disruption of Pin1-Nanog interaction suppresses embryonic stem cells' capability to self-renew and form teratomas [95].

The fact that Pin1 is indispensable for the self-renewal and maintenance of pluripotent stem cells is also demonstrated by its regulation of phosphorylated Oct4. Pin1 interacts with the phosphorylated Ser12-Pro motif of Oct4 and increases Oct4 stability and transcriptional activity. The induction of induced pluripotent stem (iPS) cells upregulates Pin1 expression, which in turn further enhances the frequency of iPS cell generation. Inhibition of Pin1 activity significantly suppresses the colony formation and induces the aberrant differentiation of human iPS cells and murine ES cells [94]. Thus, Pin1 promotes the induction and maintenance of pluripotency through its regulation of STAT3, Nanog and Oct4.

Pin1 regulates nuclear receptors

Estrogen receptor-α (ERα), a nuclear receptor expressed in breast epithelial cells, functions as a hormone-regulated transcription factor. Activation of the PI3K pathway promotes the nuclear translocation of CDK2 and phosphorylation of CDK2 at T160 in activation site [97]. Activated CDK2 in turn phosphorylates ERα at S294 and mediates Pin1-ERα interaction, which increases ERK-dependent ERα phosphorylation at S118 and S167 in the transcriptional activation function domain and promotes ERα dimerization and activity [98, 99]. Pin1 has also been shown to bind to ERα pS118 directly and cause the *cis-trans* isomerization of the pS118-P119 bond of ERα. This isomerization stabilizes ERα protein by blocking ERα interaction with the E3 ligase, E6AP, and thus inhibiting the E6AP-mediated ubiquitylation and degradation of ERα. In addition, Pin1 and ERα levels are positively correlated in human breast carcinoma specimens [100, 101].

Pin1 also promotes the activation of nuclear receptor-regulated transcription by interacting selectively with phosphorylated steroid receptor coactivator 3 (SRC-3/AIB1/pCIP) and modulating interactions between SRC-3 and cyclic adenosine monophosphate response element binding protein (CREB)-binding protein (CBP)/p300 coactivators [102].

Pin1 regulates transcription factors and co-regulators involved in tumor suppression and growth inhibition

In addition to transcription regulators that promote tumorigenesis, Pin1 also regulates a large number of tumor suppressors and growth inhibitors, including Smad transcription factors, the co-repressor silencing mediator for retinoic acid and thyroid hormone receptor (SMRT), forkhead box O (FOXO), promyelocytic leukemia protein (PML), runt-related transcription factor 3 (RUNX3), the retinoblastoma protein (pRb), p53 and p73 [3, 103-105]. Disruption of transforming growth factor-β (TGF-β) signaling, which is crucial in numerous cellular processes including proliferation, differentiation, migration and apoptosis often occurs in cancer. Pin1 interacts with Smad2 and Smad3. Phosphorylation of (S/T)Pro motifs (T179, S204, S208 and S213) in the Smad3 linker region enhances the interaction between Pin1 and Smad2/3, and the interaction of Smad2/3 with the ubiquitin E3 ligase Smurf2, which leads to enhanced Smad ubiquitylation and proteasomal degradation, thus inhibiting TGF-β-induced transcription and gene expression [106].

SMRT is a transcriptional co-repressor that participates in diverse signaling pathways and is involved in human diseases. Her2 activation decreases SMRT stability in a CDK2- and Pin1-dependent manner. CDK2 phosphorylates SMRT at multiple sites (including S1241 and S1469). SMRT phosphorylation, which is required for Pin1 binding, decreases SMRT stability, thereby affecting SMRT-dependent transcriptional repression. Tamoxifen treatment of breast cancer cells repressed the expression of both c-Myc and the progesterone receptor; knockdown of SMRT expression compromised this repression, whereas depletion of Pin1 or CDK2 enhanced the repression of both genes [107].

The FOXO protein family, an evolutionarily conserved subclass of transcription factors, is a family of tumor suppressors. Oxidative stress induces the binding of Pin1 to FOXO4 phosphorylated at multiple S/T residues and attenuates FOXO4 monoubiquitylation, in a manner mediated by the deubiquitylating enzyme HAUSP/USP7. Pin1-dependent deubiquitylation inhibits the nuclear translocation of FOXO4 and decreases its transcriptional activity towards target genes, including the cell cycle arrest gene *CDKN1B* (encoding p27Kip1). Notably, p27Kip1 expression levels are inversely correlated with Pin1 expression in human primary breast cancer specimens [108], further supporting the idea that Pin1 suppresses p27 expression by inhibiting FOXO transcriptional activity. Pin1 has also been shown to directly bind to p27Kip1 at pT187, which is phosphorylated by cyclin E-CDK2. Pin1 catalyzes the *cis-trans* isomerization of p27 and increases p27 stability through regulating its polyubiquitylation [109, 110].

The nuclear PML, functioning as a tumor suppressor, regulates apoptosis, viral infection, senescence, DNA damage repair and cell cycle progression. PML protein expression is greatly reduced in many types of cancer cells [111], and $PML^{-/-}$ mice are prone to developing tumors in chemical and physical models of carcinogenesis [112]. The stability of PML is reduced through its interaction with Pin1, in which the four key serine residues (S403, S505, S518 and S527) in the C-terminal half of PML that are phosphorylated in mammalian cells bind to Pin1. ERK2 was identified as a protein kinase phosphorylayting S403 and S505 of PML. SUMOylation of PML blocks the interaction between Pin1 and PML, thus preventing the degradation of PML. The Pin1-mediated degradation of PML protects breast cancer cells from hydrogen peroxide-induced death and increases their proliferation rates [113, 114].

RUNX3 functioning as a tumor suppressor is frequently deleted or transcriptionally silenced in cancer. RUNX3 encodes a member of the runt domain-containing family of transcription factors. RUNX3 and a β-subunit form a complex that binds to the core DNA sequence 5′-YGYGGT-3′, which is found in a number of enhancers and promoters, and this complex can either activate or sup-

press transcription [104, 115]. Pin1 interacts with four phosphorylated Ser/Thr-Pro motifs (T209, T212, T231 and S214) in RUNX3 via its WW domain, which results in the ubiquitylation and proteasomal degradation of RUNX3 and suppression of its transcriptional activity [104].

pRb represses gene expression, and hyperphosphorylation of pRb releases its inhibition of E2F transcription factors. Pin1 directly interacts with CDK-phosphorylated S608/612 in the spacer domain of pRb, which enables the interaction between CDK/cyclin complexes and pRb in middle and late G1 phase and promotes pRb S780 phosphorylation. pRb deficiency abrogates the Pin1 deficiency-induced inhibitory effect on cell proliferation, further supporting the hypothesis that pRB is a substrate of Pin1 [105, 116].

The tumor suppressor p53 is important in cell cycle arrest or apoptosis in response to a variety of stimuli. In response to DNA damage, p53 interacts with Pin1, and this interaction is dependent on p53 phosphorylation at S33, T81 and S315. The binding of Pin1 to p53 elicits conformational changes in p53 and stimulates its DNA-binding activity and transactivation function towards the *p21* promoter. Moreover, Pin1-deficient cells are defective in p53 activation and the timely accumulation of p53 and have impaired checkpoint control in response to DNA damage [117-119]. However, a p53 knock-in mouse model in which the MAPK-phosphorylated and Pin1-targeted T76 and T86 residues in p53 were mutated to Ala, showed only a moderate decrease in p53 stability and no obvious defects in p53's transactivation or tumor suppressor function [120, 121]. These results suggest that Pin1-regulated isomerization of the T76-P and T86-P motifs is not instrumental in p53's transcriptional activity. The development of a mouse model for testing the effect of mutations of other potential PIN1-binding sites on p53 function is warranted.

In response to treatment with doxorubicin and etoposide, Pin1 induces the mitochondrial translocation and apoptotic activity of p53, and this translocation is dependent on the homeodomain interacting protein kinase 2 (HIPK2)-mediated phosphorylation of p53 at S46 [122]. Thus, Pin1 regulates both nuclear and mitochondrial p53 functions in cellular response to genotoxic stress [117-119]. In tumor cells lacking functional p53, chemotherapeutic drug treatment enhances the c-Abl-dependent phosphorylation of the p53-related protein, p73 by p38 MAPK at S412, T442 and T482, which promotes the binding of p73 to Pin1 for p73's subsequent acetylation by p300. Acetylation of p73 increases its protein stability and transcriptional activity, and promotes apoptosis [123].

The physiological relationship between Pin1 and p53 was also demonstrated using $Pin1^{-/-}p53^{-/-}$ mice, which showed that development of lymphomas in p53-deficient mice was inhibited by Pin1 ablation. In addition, Pin1 plays an important role in mutant p53-promoted transformation and metastasis. Pin1 promotes the mutant p53-dependent inhibition of the anti-metastasis factor p63 and promotes the induction of a mutant p53 transcriptional program to increase tumor aggressiveness. Pin1 also cooperates with mutant p53 in Ras-dependent transformation to enhance tumorigenesis. Furthermore, in breast cancer patients, the combination of Pin1 overexpression and p53 mutation is an independent prognostic factor for poor clinical outcome [124]. These results suggest that Pin1 impacts p53 function at multiple levels [24].

Pin1 directly regulates proteins involved in apoptosis

Pin1 regulates cell apoptosis by directly regulating pro-apoptotic proteins such as B-cell lymphoma 2 (Bcl-2)-associated X protein (BAX) and death-associated protein 6 (Daxx) or anti-apoptotic proteins such as Bcl-2 and myeloid cell leukemia-1 (Mcl-1) [3, 103]. Upon stimulation with granulocyte-macrophage colony-stimulating factor, activated ERK1/2 phosphorylates BAX at T167. This phosphorylation facilitates an interaction between BAX and Pin1. Pin1 inhibition leads to the cleavage and mitochondrial translocation of BAX, as well as caspase activation and cell apoptosis [125]. Upon oxidative stress, Daxx is phosphorylated at the Ser178-Pro motif, promoting Pin1 binding. Pin1 overexpression results in the rapid degradation of Daxx via the ubiquitin-proteasome pathway, and this degradation may be mediated by a Cul3-based ubiquitin ligase. Depletion of Pin1 significantly enhances the apoptotic response induced by hydrogen peroxide or stimulatory anti-Fas antibodies. In addition, in human glioblastoma, Pin1 expression levels inversely correlate with the degree of Daxx nuclear accumulation, supporting that Pin1 plays an important role in the negative regulation of Daxx [126, 127].

Bcl-2 is an anti-apoptotic oncoprotein. Microtubule-damaging drug-induced phosphorylation of Bcl-2 predominantly occurs at S70 and S87 and is detrimental to the protein's anti-apoptotic function. Phospho-Bcl-2 translocates into the nucleus and interacts with Pin1. Longer Taxol treatment causes proteasomal degradation of Pin1; although Pin1 degradation facilitates the dephosphorylation of Bcl-2, it cannot rescue cells from the already committed apoptotic process [128]. Mcl-1, a Bcl-2-like anti-apoptotic protein involved in chemoresistance in several types of human cancer, is also regulated by Pin1. Activated ERK1/2 phosphorylates Mcl-1 at T92

and T163, and this phosphorylation is required for the association of Mcl-1 and Pin1, which results in the stabilization of Mcl-1. Inhibition of ERK or depletion of Pin1 sensitizes breast cancer cells to paclitaxel treatment [129]. The anti-apoptotic effect of Pin1 is also related to its regulation of survivin. Pin1 has been found to interact with the CDC2-phosphorylated Thr34-Pro35 motif of survivin and enhance the association of phosphorylated survivin with pro-caspase-9 and hepatitis B X-interacting protein. Pin1 overexpression, which suppresses caspase-3 and caspase-9 activity, has been detected in hepatocellular carcinoma and may play an important role in hepatocarcinogenesis [130]. Thus, Pin1 promotes cell survival by enhancing the function of anti-apoptotic proteins and inhibiting that of pro-apoptotic proteins.

Targeting Pin1 for cancer treatment

Compelling data suggest that Pin1 can be targeted to treat human cancer. A number of potent Pin1 antagonists have been developed to either inhibit the PPIase activity of Pin1 or target the Pin1 WW domain to prevent the binding of Pin1 to its substrates [131].

PPIase domain inhibitors identified from enzymatic or cell-based assays include Juglone, PiB [131], dipentamethylenethiuram monosulfide [132] and the halogenated phenylisothiazolone TME-001 [133]. Although these compounds inhibit or inactivate Pin1, questions regarding the specificity of these compounds have been raised, as they can inhibit both the proliferation of Pin1-deficient MEFs and the activity of other cellular targets such as cyclophilin A (which is also inhibited by TME-001) [131]. The use of solid-phase peptide library synthesis and screening of cyclic peptide libraries have revealed that peptidic PPIase inhibitors such as Ac-Phe-DThr(PO3H2)-Pip-Nal-Gln-NH2 and cyclo(D-Ala-Sar-D-Thr(PO3H2)-Pip-Nal-Tyr-Gln)-Lys-SH can bind to the Pin1 PPIase domain and inhibit Pin1 activity [131]. Other high-affinity inhibitory peptides such as Ac-Phe1-L-(or D-)P.Thr2-Pip3-Nal4 (naphthylalanine)-Gln5-NH_2 and Ac-Phe-D-Thr(PO3H2)-Pip-Nal-Gln-NH2, the latter of which specifically blocks HeLa cells in the G2/M phase, were also developed [134, 135]. In addition, small molecules have been derived using structure-based drug design, leading to the development of single-digit-nanomolar Pin1 inhibitors, but, unfortunately, with poor cell permeability [131].

Pin1 WW domain inhibitors bind to the Pin1 WW domain to prevent the binding of Pin1 to its substrates. Epigallocatechin gallate (EGCG), a cancer chemopreventive compound and the major flavonoid in green tea, may bind to both the WW domain and the PPIase domain. The interaction of EGCG with the Pin1 WW domain involves amino acids implicated in peptide binding and thus should interfere with WW-domain-mediated substrate binding. The binding site for EGCG in the PPIase domain is distinct from the central active site, which suggests that EGCG's inhibition of Pin1 likely occurs via the WW domain [131].

Although a number of potent Pin1 antagonists have been developed, systematic preclinical and clinical studies are needed to evaluate safety and the effect of Pin1 inhibitors on tumor development. Because Pin1 is positioned to play a general regulatory role in proline-directed kinase signaling and *Pin1* knockout mice exhibit defects in breast development in pregnancy and later have retinal degeneration and neurodegenerative conditions in brain [80, 92], Pin1 inhibitors could have many broad-ranging effects. However, since drugs never inhibit their targets completely *in vivo*, there may be a therapeutic window where Pin1 inhibitor drugs could be effective, particularly because tumors with elevated levels of Pin1 are likely to be more dependent on Pin1 activity than normal cells in the body. Some unwanted neurodegenerative side effects might be alleviated by designing Pin1 inhibitors that fail to cross the blood brain barrier. The successful specific inhibition of Pin1 and its regulators in tumors by compounds or other approaches may offer a new route to efficiently eliminate cancer.

Conclusion

Accumulating evidence demonstrates that Pin1 is regulated by different mechanisms at the transcriptional and posttranslational levels and that Pin1 overexpression is correlated with poor prognosis in several types of human cancers. In addition, the Pin1-mediated *cis-trans* isomerization of a wide variety of phosphorylated proteins precisely regulates signal transduction from proteins at the plasma membrane to transcription factors and regulators. Thus, Pin1-mediated *cis-trans* isomerization has profound regulatory effects on many cellular activities, including metabolism, mobility, cell cycle progression, proliferation, survival and apoptosis. The instrumental functions of Pin1 in tumor development make Pin1 a potential target for cancer treatment.

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