Prolyl isomerase Pin1 as a molecular target for cancer diagnostics and therapeutics

Kun Ping Lu*

Cancer Biology Program, Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215
*Correspondence: klu@bidmc.harvard.edu

Introduction

Cancer is a collection of diseases whose common feature is uncontrolled cell proliferation. The phosphorylation of proteins on serine or threonine residues that immediately precede a proline (pSer/Thr-Pro), so-called Pro-directed phosphorylation, is a central signaling mechanism controlling normal cell proliferation and malignant transformation (Blume-Jensen and Hunter, 2001; Lu et al., 2002b). Ser/Thr-Pro motifs are the exclusive phosphorylation sites for a large number of Pro-directed protein kinases that play essential roles in signal transduction and cell cycle progression. Furthermore, many oncogenes and tumor suppressors themselves are directly regulated by Pro-directed phosphorylation and/or can trigger signaling pathways involving Pro-directed phosphorylation.

Significant progress has been made in elucidating the mechanisms controlling Pro-directed phosphorylation and its physiological function and pathological importance. However, little is known about how most Ser/Thr phosphorylation events regulate protein function, although phosphorylation-induced conformational changes have been proposed to play a significant regulatory role. The recent identification and characterization of a peptidyl-prolyl *cis*/*trans* isomerase (PPIase), Pin1, which specifically regulates the conformation of specific Pro-directed phosphorylation sites in certain proteins, has led to the discovery of a new postphosphorylation regulatory mechanism (Lu et al., 1996, 2002b; Ranganathan et al., 1997; Yaffe et al., 1997). In this mechanism, Pin1 binds to and isomerizes specific pSer/Thr-Pro motifs and catalytically induces conformational changes following phosphorylation. Such conformational changes can have profound effects on the function of many Pin1 substrates, thereby playing an important role in many cellular events, such as cell cycle progression, transcriptional regulation, RNA processing, and cell proliferation and differentiation (Lu et al., 2002b).

Notably, Pin1 is not only overexpressed in a large number of human cancers, but also is an excellent prognostic marker in some cancers (Ayala et al., 2003; L. Bao et al., submitted; Ryo et al., 2001; Wulf et al., 2001). Furthermore, Pin1 overexpression can function as a critical catalyst that amplifies multiple oncogenic signaling pathways during oncogenesis (Liou et al., 2002; Ryo et al., 2001, 2002, 2003; Wulf et al., 2001). Significantly, inhibition of Pin1 in cancer cells via multiple approaches triggers apoptosis or suppresses transformed phenotype (Lu et al., 1996; Rippmann et al., 2000; Ryo et al., 2002). In addition, compounds that may selectively inhibit Pin1 are being identified (Hennig et al., 1998; Uchida et al., 2003). These results suggest that Pin1-mediated postphosphorylation regulation may provide a unique opportunity for disrupting oncogenic pathways and represent an appealing target for novel anticancer therapies. The primary focus of this review is to discuss these promising features of Pin1 as a new molecular target for cancer diagnostics and therapeutics.

Pro-directed phosphorylation is a central mechanism to control cell proliferation and transformation

Ser/Thr-Pro motifs are the exclusive phosphorylation sites for many key protein kinases involved in cell growth control (Lu et al., 2002b). These include all cyclin-dependent kinases (CDKs), which control cell cycle transitions, as well as most, if not all, the mitogen-activated protein kinases (MAPKs), and glycogen synthase kinase 3β (GSK-3β), which plays key roles in cell signaling (Figure 1A). Importantly, the ability of these kinases to phosphorylate the substrates strictly depends on the presence of a Pro residue that immediately follows Ser/Thr; its point mutation abolishes phosphorylation and typically disrupts phosphorylation signaling, indicating an essential role of the Pro residues. Pro residues have the unique property of existing in two completely distinct isomers, *cis* and *trans*, in folded proteins and therefore can provide a potential backbone switch in the polypeptide chain that is controlled by *cis*/*trans* isomerization around the peptidyl-prolyl bond (Figure 1A) (Hunter, 1998; Lu et al., 2002b).

This intrinsically rather slow conversion can be catalyzed by *cis*/*trans* peptidyl-prolyl isomerases (PPIases), which play an important role in protein folding or refolding (Hunter, 1998). Cyclophilins and FK506 binding proteins (FKBPs) are two well-characterized families of PPIases, because they act as cellular receptors for clinically important immunosuppressive drugs. However, the PPIase activity of these proteins is neither responsible for the drug action in the immune system nor essential for cell survival in yeast (Hunter, 1998). Therefore, evidence for the biological importance of PPIase activity in these proteins has been elusive. Notably, Pro-directed phosphorylation further restrains the already slow spontaneous isomerization of peptidyl-prolyl bonds (Lu et al., 2002b; Yaffe et al., 1997). Moreover, Pro-directed phosphorylation renders the peptide bond resistant to the catalytic action of cyclophilins and FK506 binding proteins (FKBPs) (Hunter, 1998; Lu et al., 2002b; Yaffe et al., 1997). Therefore, there is a need for a different enzyme to isomerize the Pro-directed phosphorylated peptide bonds.

Pin1 catalyzes conformational changes in certain key Pro-directed phosphorylation sites and functions as a pivotal catalyst for oncogenesis

To date, Pin1 is the only enzyme known to specifically isomerize pSer/Thr-Pro bonds (Figure 1A) (Ranganathan et al., 1997; Yaffe et al., 1997). Pin1 is highly conserved and its budding yeast homolog Ess1 is the only known essential gene out of total 13 known PPIase genes present in the yeast genome (Lu et al., 1996). The striking substrate specificity of Pin1 toward

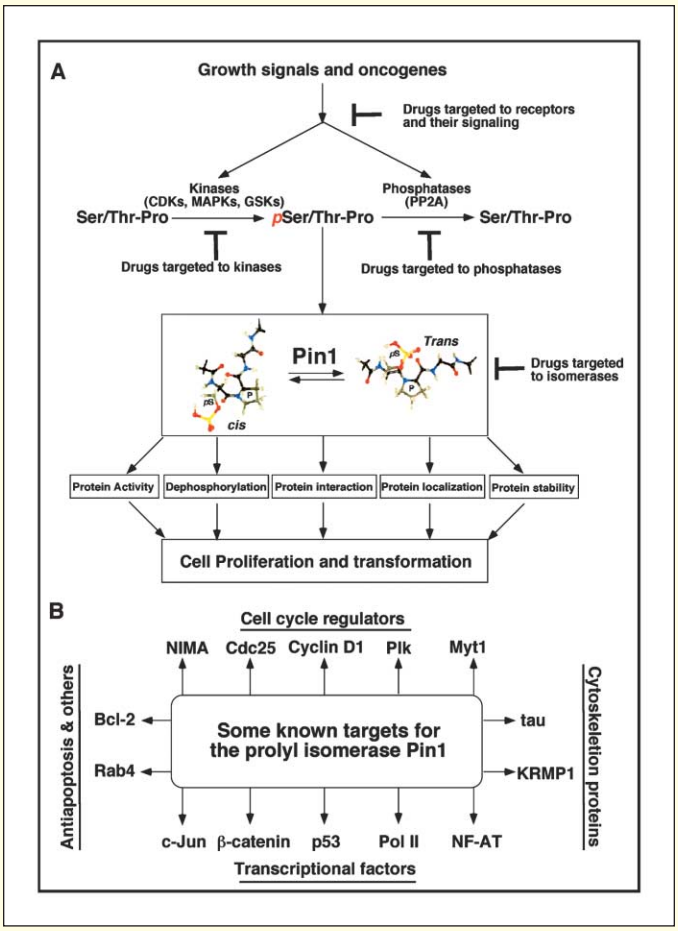

Figure 1. Pin1 is a novel postphosphorylation regulator in signal transduction

In response to growth signals or oncogenic activation, a subset of proteins are phosphorylated on certain regulatory Ser/Thr-Pro motifs and become the substrate for Pin1, which alters the conformation of proteins by catalyzing either the *trans* to *cis* or the *cis* to *trans* isomerization of pSer/Thr-Pro depending on specific target sites. The results so far support the notion that the conformational changes following phosphorylation are essential for controlling the function of at least certain Pin1 substrates.

certain pSer/Thr-Pro bonds results from its unique two-domain structure consisting of an N-terminal WW domain and a C-terminal PPIase domain that form a double-check mechanism (Ranganathan et al., 1997; Yaffe et al., 1997; Zhou et al., 2000). The WW domain of Pin1 binds only to specific pSer/Thr-Pro motifs, which are often critical regulatory phosphorylation sites in Pin1 substrates (Lu et al., 1999a, 1999b, 2002b; Ryo et al., 2001; Shen et al., 1998; Wulf et al., 2001; Zhou et al., 2000). This WW domain binding targets the Pin1 catalytic domain close to its substrates, where the PPIase domain isomerizes specific pSer/Thr-Pro motifs and catalytically induces conformational changes (Lu et al., 1999b; Zhou et al., 2000). The residues in the Pin1 catalytic site that are responsible for the phosphorylation specificity are a pair of highly conserved Arg residues that are not found in other known PPIases (Ranganathan et al., 1997; Yaffe et al., 1997).

Although protein kinases and phosphatases are *trans* specific (Weiwad et al., 2000; Zhou et al., 2000), both *cis* and *trans* pSer/Thr-Pro bonds are likely present in folded phosphoproteins. Out of about 10^5 Ser/Thr-Pro bonds in sequence databases, only ~1% Ser/Thr-Pro bonds can be found in the protein structure database, and the propensity of *cis* Ser/Thr-Pro bonds is in the range of 10%–25%. Since phosphorylation on Ser/Thr-Pro motifs in peptides does not greatly affect the final equilibrium ratio of *cis* and *trans* prolyl bonds, the probability of pSer/Thr-Pro bonds in the *cis* conformation is estimated to be 10%–20% (Zhou et al., 2000). Given that phosphorylation does drastically reduce the isomerization rate of the pSer/Thr-Pro bonds, Pin1 would be needed to accelerate either the *trans* to *cis* or the *cis* to *trans* isomerization, depending on specific target sites, and induce conformational changes in proteins to regulate their function (Figure 1A). Indeed, Pin1 has been shown to regulate the catalytic activity, phosphorylation status, protein-protein interactions, subcellular location, and/or protein stability of its substrates. These substrates include many essential cell cycle regulators, oncogenes, and tumor suppressor proteins known to play important roles in cancer (Figures 1A and 1B) (Liou et al., 2002; Lu et al., 1999b; Ryo et al., 2001; Shen et al., 1998; Wulf et al., 2001; Zhou et al., 2000). Thus, Pin1-induced conformational change is a new signaling mechanism that controls protein function after phosphorylation (Lu et al., 2002b).

In addition to the availability of specific phosphorylated substrates, Pin1 function is normally regulated tightly through multiple mechanisms, including transcriptional regulation and posttranslational modifications. Pin1 expression is activated by the E2F family of transcription factors in response to growth signaling, such as activation of Ras or Neu (Ryo et al., 2002). Like many other E2F target genes (Nevins, 2001), Pin1 transcription and its protein levels fluctuate during cell cycle progression in nontransformed cells (Ryo et al., 2002), but are constitutively elevated in transformed cells (Shen et al., 1998). Pin1 function is also regulated by protein phosphorylation of itself. Although the specific kinases and phosphatases involved remain to be identified, cell cycle-specific phosphorylation of the Pin1 WW domain inhibits its ability to bind substrates and to regulate the subcellular localization of Pin1 (Lu et al., 2002b).

The significance of Pin1 regulation is highlighted by the findings that Pin1 is prevalently overexpressed in human cancers and also functions as a critical catalyst at multiple steps in oncogenic signaling pathways (Figure 2). For example, in breast cancer, Pin1 is drastically overexpressed and mainly exists in a hypophosphorylated, presumably active form in a large number of human breast cancer tissues (Wulf et al., 2001). More importantly, Pin1 regulates the function of cyclin D1, an essential protein in breast cancer development (Yu et al., 2001), through multiple mechanisms (Ryo et al., 2003). Pin1 collaborates with Ras/JNK signaling to increase the transcriptional activity of Jun toward cyclin D1 (Figure 2B) (Wulf et al., 2001). It also activates β-catenin by preventing its binding to the tumor suppressor APC, which leads to induction of β-catenin downstream target genes such as cyclin D1, Jun, and Myc (Figure 2B) (Ryo et al., 2002). Furthermore, Pin1 can directly act on cyclin D1 to increase its stability (Liou et al., 2002) (Figure 2B). In addition, Myc can enhance cyclin D1 function by inducing Cdk4 expression and also directly induce *E2F* family genes (Nevins, 2001). These molecules act synergistically to regulate cyclin D1 function. Indeed, loss of Pin1 function in the mouse causes failure of the breast epithelial compartment to undergo the massive proliferative changes associated with pregnancy, the major phenotype in cyclin D1 null mice (Liou et al., 2002). Finally, *Pin1* itself is an E2F target gene, which can be further upregulated by E2F

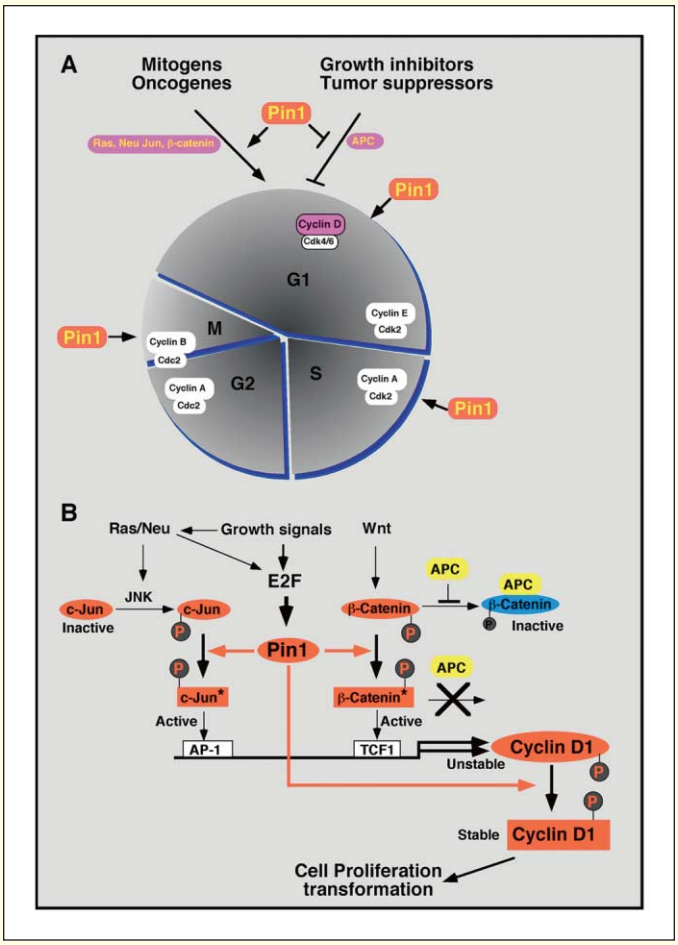

Figure 2. Pin1 functions as a pivotal catalyst for integrating multiple oncogenic signaling pathways
A: Pin1 is a downstream target of growth factors and functions at multiple steps in growth signal transduction as well as in eventual cell cycle progression.
B: A specific example is given of how Pin1 controls expression and post-translational stabilization of cyclin D1 in collaboration with other oncogenes in breast cancer.

activation in a positive feedback loop involving cyclin D1/Cdks, E2F, and Rb, as well as Pin1 (Ryo et al., 2002) (Figure 2B). This positive-feedback mechanism may play a central role in aberrant cell proliferation and oncogenesis. In fact, Pin1 overexpression in normal mammary epithelial cells can confer anchorage-independent cell growth and other early transformed properties (Ryo et al., 2002). Moreover, Pin1 overexpression can also greatly enhance transformed phenotypes induced by oncogenic Neu and Ras (Ryo et al., 2002). In contrast, inhibition of Pin1 dramatically reduces both cell proliferation and transformation induced by oncogenic Neu and Ras. This reduction can be reversed by expression of the constitutively active cyclin D1-T286A mutant that is resistant to Pin1 inhibition (Ryo et al., 2002). These results indicate that cyclin D1 is a specific downstream target of Pin1 in the transformation of mammary epithelial cells.

During cancer development, multiple oncogenic signaling pathways are often activated, which eventually lead to Pro-directed phosphorylation and uncontrolled cell proliferation (Blume-Jensen and Hunter, 2001). The above results have led us to propose that Pin1 would cooperate with these multiple oncogenic pathways to promote cell proliferation and transformation (Ryo et al., 2003). In this model, Pin1 responds to and amplifies oncogenic signals, functioning as an indispensable translator and amplifier of oncogenic signaling pathways. A crucial genetic test for this hypothesis will be to study tumor development in Pin1-transgenic or Pin1-deficient mice in the presence or absence of various oncogenes.

Pin1 is prevalently overexpressed in human cancers and is a molecular target for cancer diagnostics

Overexpression of Pin1 was originally observed in human breast cancer tissues and breast cancer cell lines (Ryo et al., 2001; Wulf et al., 2001). This observation has been confirmed and expanded by a large scale of study comparing Pin1 expression in several dozen different human tumor types and their corresponding normal tissues. Pin1 overexpression is observed in most commonly encountered cancers such as prostate, breast, cervical, brain, lung, and colon cancer, but not in others (L. Bao et al., submitted). These results indicate that Pin1 overexpression is a prevalent and specific event in human cancers. Since *Pin1* is an E2F target gene (Ryo et al., 2002), deregulation of the Rb/E2F pathway, which is found in many human cancers (Nevins, 2001), likely plays a significant role in increased Pin1 expression in human cancers. In addition, it would be interesting to determine whether any genetic mutations or amplifications might be present in human cancers, which might contribute to upregulation of Pin1 function in cancer by increasing expression or abolishing the inhibitory phosphorylation. Any such changes would provide strong genetic evidence for a critical role of Pin1 in oncogenesis.

Given prevalent overexpression of Pin1 in human cancers (L. Bao et al., submitted; Ryo et al., 2001; Wulf et al., 2001), detection of Pin1 expression might help distinguish cancer cells from normal cells as well as identify cancer patients for possible Pin1-based cancer therapies. Furthermore, it appears that determination of Pin1 expression might have an important prognostic value for managing cancer patients. In breast cancer, elevated Pin1 expression strongly correlates with upregulation of β-catenin (Ryo et al., 2001). Given that β-catenin is a strong and independent prognostic factor (Lin et al., 2000), these results suggest that Pin1 may be of significant prognostic value in oncology.

This notion has been recently supported by a large scale study correlating Pin1 expression with the clinic outcome of 580 prostate cancer patients who underwent radical prostatectomy by a single surgeon and did not have any preoperative adjuvant therapy (Ayala et al., 2003). Prostate cancer is the most common male cancer in the United States, and radical prostatectomy is a definitive form of therapy for clinically localized prostate cancer. However, approximately one-third of the patients treated with radical prostatectomy experience progression even when tumors are confined pathologically to the prostate. Accurate prediction of the risk of recurrence would be valuable when considering early adjuvant therapy or some form of investigational treatments. Currently, there is no validated prognostic marker capable of reliably distinguishing between groups of patients who could be safely entered into watchful waiting protocols versus patients who require, and would benefit from, definitive surgery/radiation therapy. Thus, a substantial part of prostate cancer research aims to define

accurate prognostic markers for estimating malignant potential, with the majority focusing on gene expression profiling (Dhanasekaran et al., 2001; Singh et al., 2002).

Pin1 is overexpressed in prostate cancer cells both in the intensity of expression and the percentage of Pin1-positive cells based on immunocytochemistry (Ayala et al., 2003). More importantly, Pin1 expression positively correlates with lymph node metastasis and clinical recurrence (Ayala et al., 2003). Pin1 expression is determined by visual evaluation to be positive or negative, and its positive expression is a strong independent predictor of recurrence in prostate cancer patients after radical prostatectomy, which is determined by the reappearance of PSA (prostate cancer specific antigen) (Ayala et al., 2003). The predictive value of Pin1 expression becomes even more obvious when Pin1 expression is semiquantitatively evaluated by automated imaging analysis. This imaging analysis can further discriminate Pin1 staining intensity as well as the sum index of Pin1 expression that is defined as the sum of the highest intensity of Pin1 staining and the average percentage of Pin1-positive cells (Ayala et al., 2003). A patient with a high Pin1 sum index has a >8 times greater risk of recurrence than a patient with a low expression index. Furthermore, Pin1 is also an excellent predictor of recurrence in the subset of patients with a Gleason score of 6 or 7, where it is almost impossible to predict clinic outcome (Ayala et al., 2003). Again, a patient with high Pin1 expression again has over 8 times the risk of having earlier recurrence than one with low Pin1 expression. Moreover, Pin1 outperforms other known and currently used clinicopathologic parameters, including lymph node metastasis, preoperative PSA levels, Gleason score, surgical margins, seminal vesicle status, and extracapsular extension (Ayala et al., 2003). Although it is important to confirm these results using other independent sets of prostate cancer samples, these results show that Pin1 expression is at least as good as, if not better than, currently used postoperatively available clinicopathologic parameters. It could potentially be utilized in the preoperative setting to assist in choice of treatment. In addition, a test for Pin1 expression in tumor tissues after radical prostatectomy might help identify patients who will need more aggressive therapy.

Pin1 is an attractive molecular target for cancer therapeutics

In the last several years, signal transduction cascades have become promising targets for anticancer therapy (Druker, 2002). For example, trastuzumab, the monoclonal antibody against Her2/neu, is now part of the standard armamentarium in treating Her2/neu-overexpressed breast cancer patients (Harries and Smith, 2002), and imatinib, a specific inhibitor of the Bcr-Abl tyrosine kinase, has been successfully used to treat chronic myeloid leukemia (Deininger et al., 2003). A number of features make Pin1 an attractive new drug target for cancer therapy. First, Pin1 is an enzyme with an extraordinarily high substrate specificity and well-defined active site (Lu et al., 1999b; Ranganathan et al., 1997; Shen et al., 1998; Yaffe et al., 1997). Historically, it has been much easier to develop inhibitors specific for an enzyme than for a nonenzymatic protein. Second, Pin1 is prevalently overexpressed in human cancers, and its expression levels correlate with the poor clinical outcome (Ayala et al., 2003; L. Bao et al., submitted; Ryo et al., 2001; Wulf et al., 2001). Third, Pin1 functions as the critical catalyst for multiple oncogenic pathways; its overexpression is able to confer transforming properties on normal cells and to enhance transformed phenotype induced by other oncogenes such as Neu and Ras (Liou et al., 2002; Ryo et al., 2003; Ryo et al., 2001; Ryo et al., 2002; Wulf et al., 2001). Fourth, inhibition of Pin1 using antisense *PIN1* or dominant negative Pin1 mutants causes cancer cells to enter mitotic block and apoptosis in transient transfection (Lu et al., 1996, 2002a; Rippmann et al., 2000) or suppresses the transformed phenotypes induced by Ras/Neu (Ryo et al., 2002). Furthermore, this suppression of transformed phenotypes by inhibition of Pin1 can be reversed by a constitutively active cyclin D1 mutant that is resistant to Pin1 inhibition (Ryo et al., 2002). Fifth, Pin1 knockout mice develop normally to adulthood (Fujimori et al., 1999; Liou et al., 2002), indicating that an anti-Pin1 therapy might not have general toxic effects. It is also worth pointing out that Pin1 knockout mice do develop several age-dependent phenotypes, including testicular atrophy, retinal atrophy, and neurodegeneration (Liou et al., 2002, 2003). Given that these phenotypes occur only after a long-term loss of Pin1, a short-term treatment with Pin1 inhibitors during cancer therapy is unlikely to have acute side effects, especially if the inhibitors are not readily absorbed through the blood-brain and blood-testis barriers. Finally, another beneficial feature of Pin1 inhibitors would be that they may simultaneously inhibit multiple oncogenic signaling pathways, on which cancer cells depend for growth and survival (Liou et al., 2002; Ryo et al., 2001, 2002, 2003; Wulf et al., 2001). These inhibitory activities could perhaps circumvent the characteristic genetic plasticity that has allowed cancer cells to eventually evade the toxic effects of most molecularly targeted agents.

Although Pin1 inhibition with antisense strategies and dominant-negative mutants has been employed successfully in vitro (Lu et al., 1996, 2002a; Rippmann et al., 2000; Ryo et al., 2002), the feasibility of therapeutic Pin1 inhibition has not yet been explored, largely due to the lack of highly specific inhibitors. In contrast to cyclophilins and FK506 binding proteins, where highly specific inhibitors are well characterized and widely used clinically (Hunter, 1998), the only known Pin1 inhibitors are the natural product Juglone (Hennig et al., 1998) and the small molecule PiB and its derivatives (Uchida et al., 2003). Juglone covalently inactivates a unique cysteine residue in the active site of Pin1-type and parvulin-type isomerases. Juglone has some anticancer activity and has been used as a Pin1 inhibitor in several studies in vitro (Chao et al., 2001; Rippmann et al., 2000). However, given that Juglone potently inhibits many other proteins and enzymes (Chao et al., 2001; Ryo et al., 2003), it is unlikely to be Pin1-specific in the cell. Unlike Juglone, PiB and its analogs have been shown to be competitive inhibitors with low μM IC50 values (Uchida et al., 2003). Furthermore, PiB and its analogs have been shown to inhibit cell growth of Pin1-containing cell lines, but not Pin1-deficient cells (Uchida et al., 2003). These results suggest that Pin1 is an important target for these compounds and further support the idea of utilizing Pin1 inhibitors as anticancer reagents. However, similar to Juglone, PiB and its analogs also inhibit non-phosphorylation-specific prolyl isomerases such as Par14 (Hennig et al., 1998; Uchida et al., 2003), raising the concern about the specificity of these compounds. In addition, these compounds are quite weak inhibitors of Pin1. Therefore, there is an urgent need for the development of highly specific and potent Pin1 inhibitors. In fact, several pharmaceutical companies have Pin1 inhibitors at various preclinical testing stages (e.g., Pintex, www.pintexpharm.com). Consequently, it is feasible that Pin1-

specific inhibitors will be developed in the near future. Such inhibitors might be highly effective anticancer drugs alone or in combination with established chemotherapeutic drugs or procedures. For example, Pin1 inhibitors might be used to increase the sensitivity of cancer cells to irradiation therapy as Pin1 null cells are more sensitive to irradiation, partially due to the fact that Pin1 is required for DNA damage response (Wulf et al., 2002; Zacchi et al., 2002; Zheng et al., 2002). Finally, taxol also induces mitotic arrest and apoptosis via disrupting microtubule function that is likely different from that resulting from inhibiting Pin1. Therefore, Pin1 inhibitors might be used in combination with taxol as part of a novel clinical strategy for cancer therapy. Alternatively, Pin1 inhibitors might be used in combination with other drugs that are targeted to various steps of signaling pathways (Figure 1A). These combined chemotherapies may increase their effectiveness and/or reduce chemoresistance, a major clinical problem associated with current cytotoxic chemotherapies.

Conclusion and future challenges

Pin1 is a novel postphosphorylation signaling regulator, which sits at the crossroads of many signaling pathways controlling cell proliferation and transformation involving Pro-directed phosphorylation. Following phosphorylation, Pin1 catalyzed prolyl-isomerization induces conformational changes and thereby regulates the function of a number of phosphorylated proteins that play an important role during oncogenesis. Given that Pin1 is overexpressed in the majority of human cancers and its expression levels are correlated with poor clinical outcome in some cancers, Pin1 may function as a critical catalyst that potentiates multiple oncogenic signaling mechanisms during cancer development. As a result, Pin1 inhibitors should simultaneously inhibit multiple oncogenic signaling pathways, which may circumvent the characteristic genetic plasticity that has allowed cancer cells to eventually evade the toxic effects of most molecularly targeted agents. Therefore, Pin1 represents a promising new molecular target for cancer diagnostic and therapeutics. A major challenge for the future will be to further elucidate the molecular mechanisms of Pin1 deregulation during cell transformation and to determine the in vivo role of Pin1 during oncogenesis using model systems such as Pin1 knockout and Pin1 transgenic mouse models in the presence or absence of other oncogenes. A further and significant challenge will be the development of highly specific and potent Pin1 inhibitors for clinical trials and eventually cancer therapies.

Acknowledgments

I am grateful to D.G. Wang, L. Bao, A. Kimzey, J.M. Sowadski at Pintex Pharmaceuticals, and G. Sauter at University of Basel for sharing results prior to publication, and to B. Neel and T. Hunter for critically reading this manuscript. K.P.L. is a Pew Scholar and a Leukemia and Lymphoma Society Scholar. Our research cited in this review was supported by NIH grants GM56230, GM58556, and AG17870 to K.P.L.

References

Ayala, G., Wang, D., Wulf, G., Frolov, A., Le, R., Wheeler, T., Sowadski, J.M., Lu, K.P., and Bao, L. (2003). Pin1 is a novel prognostic marker in prostate cancer. Cancer Res., in press.

Blume-Jensen, P., and Hunter, T. (2001). Oncogenic kinase signalling. Nature *411*, 355–365.

Chao, S.H., Greenleaf, A.L., and Price, D.H. (2001). Juglone, an inhibitor of the peptidyl-prolyl isomerase Pin1, also directly blocks transcription. Nucleic Acids Res. *29*, 767–773.

Deininger, M.W., O'Brien, S.G., Ford, J.M., and Druker, B.J. (2003). Practical management of patients with chronic myeloid leukemia receiving imatinib. J. Clin. Oncol. *21*, 1637–1647.

Dhanasekaran, S.M., Barrette, T.R., Ghosh, D., Shah, R., Varambally, S., Kurachi, K., Pienta, K.J., Rubin, M.A., and Chinnaiyan, A.M. (2001). Delineation of prognostic biomarkers in prostate cancer. Nature *412*, 822–826.

Druker, B.J. (2002). Perspectives on the development of a molecularly targeted agent. Cancer Cell *1*, 31–36.

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

Harries, M., and Smith, I. (2002). The development and clinical use of trastuzumab (Herceptin). Endocr. Relat. Cancer *9*, 75–85.

Hennig, L., Christner, C., Kipping, M., Schelbert, B., Rucknagel, K.P., Grabley, S., Kullertz, G., and Fischer, G. (1998). Selective inactivation of parvulin-like peptidyl-prolyl cis/trans isomerases by juglone. Biochemistry *37*, 5953–5960.

Hunter, T. (1998). Prolyl isomerase and nuclear function. Cell *92*, 141–143.

Lin, S.Y., Xia, W., Wang, J.C., Kwong, K.Y., Spohn, B., Wen, Y., Pestell, R.G., and Hung, M.C. (2000). Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proc. Natl. Acad. Sci. USA *97*, 4262–4266.

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

Liou, Y.-C., Sun, A., Ryo, A., Zhou, X.Z., Yu, Z.-X., Huang, H.-K., Bronson, R., Bing, G., Li, X., Hunter, T., and Lu, K.P. (2003). Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. Nature *424*, 556–561.

Lu, K.P., Hanes, S.D., and Hunter, T. (1996). A human peptidyl-prolyl isomerase essential for regulation of mitosis. Nature *380*, 544–547.

Lu, P.J., Wulf, G., Zhou, X.Z., Davies, P., and Lu, K.P. (1999a). The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. Nature *399*, 784–788.

Lu, P.J., Zhou, X.Z., Shen, M., and Lu, K.P. (1999b). A function of WW domains as phosphoserine- or phosphothreonine-binding modules. Science *283*, 1325–1328.

Lu, P.J., Zhou, X.Z., Liou, Y.C., Noel, J.P., and Lu, K.P. (2002a). Critical role of WW domain phosphorylation in regulating its phosphoserine-binding activity and the Pin1 function. J. Biol. Chem. *277*, 2381–2384.

Lu, K.P., Liou, Y.C., and Zhou, X.Z. (2002b). Pinning down the proline-directed phosphorylation signaling. Trends Cell Biol. *12*, 164–172.

Nevins, J.R. (2001). The Rb/E2F pathway and cancer. Hum. Mol. Genet. *10*, 699–703.

Ranganathan, R., Lu, K.P., Hunter, T., and Noel, J.P. (1997). Structural and functional analysis of the mitotic peptidyl-prolyl isomerase Pin1 suggests that substrate recognition is phosphorylation dependent. Cell *89*, 875–886.

Rippmann, J.F., Hobbie, S., Daiber, C., Guilliard, B., Bauer, M., Birk, J., Nar, H., Garin-Chesa, P., Rettig, W.J., and Schnapp, A. (2000). Phosphorylation-dependent proline isomerization catalyzed by Pin1 is essential for tumor cell survival and entry into mitosis. Cell Growth Differ. *11*, 409–416.

Ryo, A., Nakamura, N., Wulf, G., Liou, Y.C., and Lu, K.P. (2001). Pin1 regulates turnover and subcellular localization of beta-catenin by inhibiting its interaction with APC. Nat. Cell Biol. *3*, 793–801.

Ryo, A., Liou, Y.C., Wulf, G., Nakamura, N., Lee, S.W., and Lu, K.P. (2002). Pin1 is an E2F target gene essential for the Neu/Ras-induced transformation of mammary epithelial cells. Mol. Cell. Biol. *22*, 5281–5295.

Ryo, A., Liou, Y.C., Lu, K.P., and Wulf, G. (2003). Prolyl isomerase Pin1, a catalyst for oncogenesis and a potential anticancer target. J. Cell Sci. *116*, 773–783.

Shen, M., Stukenberg, P.T., Kirschner, M.W., and Lu, K.P. (1998). The essential mitotic peptidyl-prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins. Genes Dev. *12*, 706–720.

Singh, D., Febbo, P.G., Ross, K., Jackson, D.G., Manola, J., Ladd, C., Tamayo, P., Renshaw, A.A., D'Amico, A.V., Richie, J.P., et al. (2002). Gene expression correlates of clinical prostate cancer behavior. Cancer Cell *1*, 203–209.

Uchida, T., Takamiya, M., Takahashi, M., Miyashita, H., Ikeda, H., Terada, T., Matsuo, Y., Shirouzu, M., Yokoyama, S., Fujimori, F., and Hunter, T. (2003). Pin1 and Par14 peptidyl prolyl isomerase inhibitors block cell proliferation. Chem. Biol. *10*, 15–24.

Weiwad, M., Kullertz, G., Schutkowski, M., and Fischer, G. (2000). Evidence that the substrate backbone conformation is critical to phosphorylation by p42 MAP kinase. FEBS Lett. *478*, 39–42.

Wulf, G.M., Ryo, A., Wulf, G.G., Lee, S.W., Niu, T., and Lu, K.P. (2001). Pin1 is overexpressed in breast cancer and potentiates the transcriptional activity of phosphorylated c-Jun towards the cyclin D1 gene. EMBO J. *20*, 3459–3472.

Wulf, G.M., Liou, Y.C., Ryo, A., Lee, S.W., and Lu, K.P. (2002). Role of Pin1 in the regulation of p53 stability and p21 transactivation, and cell cycle checkpoints in response to DNA damage. J. Biol. Chem. *277*, 47976–47979.

Yaffe, M.B., Schutkowski, M., Shen, M., Zhou, X.Z., Stukenberg, P.T., Rahfeld, J., Xu, J., Kuang, J., Kirschner, M.W., Fischer, G., et al. (1997). Sequence-specific and phosphorylation-dependent proline isomerization: A potential mitotic regulatory mechanism. Science *278*, 1957–1960.

Yu, Q., Geng, Y., and Sicinski, P. (2001). Specific protection against breast cancers by cyclin D1 ablation. Nature *411*, 1017–1021.

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

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

Zhou, X.Z., Kops, O., Werner, A., Lu, P.J., Shen, M., Stoller, G., Küllertz, G., Stark, M., Fischer, G., and Lu, K.P. (2000). Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. Mol. Cell *6*, 873–883.