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| Citation          | Wulf, Gerburg, Akihide Ryo, Yih-Cherng Liou, and Kun Ping Lu. 2003. The prolyl isomerase Pin1 in breast development and cancer. Breast Cancer Research 5(2): 76-82. |
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| Published Version | http://www.biomedcentral.com/content/pdf/bcr572.pdf                                                                                                                                               |
| Citable link      | http://nrs.harvard.edu/urn-3:HUL.InstRepos:5332801                                                                                                                                              |
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Review

The prolyl isomerase Pin1 in breast development and cancer

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Received: 8 October 2002    Revisions received: 11 December 2002    Accepted: 3 January 2003    Published: 28 January 2003

Breast Cancer Res 2003, 5:76-82 (DOI 10.1186/bcr572)
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Abstract

The prolyl isomerase Pin1 specifically isomerizes certain phosphorylated Ser/Thr-Pro bonds and thereby regulates various cellular processes. Pin1 is a target of several oncogenic pathways and is overexpressed in human breast cancer. Its overexpression can lead to upregulation of cyclin D1 and transformation of breast epithelial cells in collaboration with the oncogenic pathways. In contrast, inhibition of Pin1 can suppress the transformation of breast epithelial cells. In addition, Pin1 knockout in mice prevents massive proliferation of breast epithelial cells during pregnancy. Pin1 plays a pivotal role in breast development and may be a promising new anticancer target.

Keywords: breast cancer, breast development, cyclin D1, Pin1, phosphorylation signaling

Introduction

Phosphorylation of proteins on serine/threonine residues preceding proline (Ser/Thr-Pro) is a key signaling mechanism in breast development and breast cancer [1–3]. For example, activation of oncogenic HER2/Neu/ErbB2 and/or Ras signaling leads to activation of various Pro-directed protein kinases, which eventually enhance the transcription of the cyclin D1 gene via multiple transcription factors, including E2F, c-Jun/AP-1, and β-catenin/TCF [4–8]. Furthermore, the function of cyclin D1 itself is regulated by Pro-directed phosphorylation [9–11]. Even the events downstream from cyclin D1 are also controlled by Pro-directed phosphorylation, since cyclin D1 is a critical regulator of G1/S cyclin-dependent protein kinases, which phosphorylate only certain Ser/Thr-Pro motifs. The role of cyclin D1 in breast development and cancer has been well established. Notably, this protein is overexpressed in human breast cancer [12,13] and such overexpression can transform cells in vitro [11,14] and cause mammary hyperplasia and eventually adenocarcinomas in vivo [15]. In contrast, disruption of the cyclin D1 gene in mice suppresses massive proliferation of mammary epithelial cells during pregnancy and prevents Ha-Ras or c-Neu/HER2 from inducing breast cancer [16]. These and other results indicate that a key regulatory mechanism in breast development and cancer is Pro-directed phosphorylation. Although Ser/Thr phosphorylation has long been believed to regulate the function of proteins by altering their conformation, little has been known about the actual conformational changes and their importance until recently.

Recent identification of the new isomerase Pin1 that specifically isomerizes only the phosphorylated Ser/Thr-Pro bonds in certain proteins has led to the discovery of a new signaling mechanism, where prolyl isomerization catalytically induces conformational changes in proteins after phosphorylation to regulate protein function [17–19]. The human Pin1 gene was originally identified in a yeast genetic screen searching for proteins involved in mitotic regulation and was shown to be the first peptidylproline cis–trans isomerase (PPlase) that is essential for cell division in yeast and human cells [17]. PPlases catalyze the intrinsically rather slow cis–trans isomerization of peptide bonds N-terminal of proline residues and play a role in protein folding or refolding [3,20–22]. The two most well characterized families of PPlases are the cyclophilins and FK506-binding proteins (FKBPs), which are involved in various cell processes. Their best-known function is in the immune system, where they act as cellular receptors for the clinically relevant 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 [20–22]. Therefore, evidence for the biological and/or pathological importance of PPIase activity in these proteins has been elusive.

In contrast to all other characterized PPIases, Pin1 has a unique substrate specificity: it binds and isomerizes specific Ser/Thr-Pro motifs only after phosphorylation and thereby induces conformational changes to regulate the function of the Pin1 substrates [3,17–19]. Depending on the substrate protein, these conformational changes can affect enzymatic activity, phosphorylation status, protein–protein interactions, subcellular localization, or protein stability [3,23–29]. Functionally, Pin1-catalyzed prolyl isomerization regulates such complex processes as cell cycle progression [17,23,24,28,30–37], transcription [34–44], and the response to DNA damage [45–47]. Furthermore, Pin1 has been shown to be involved in the pathogenesis of some human diseases, such as Alzheimer’s disease [26] and cancer [34–37].

Comprehensive recent reviews on the function and regulation of Pin1 [3,27,48] and on its role in transcription [49] and in Alzheimer’s disease [50] are available. Here we review the recent studies indicating that Pin1 plays an important role in breast development and cancer and could be an attractive new target for breast cancer treatment.

**Pin1 in breast development**

The mammary gland undergoes a sequence of dynamic changes throughout the female life cycle, especially during pregnancy. These developmental changes depend on the responsiveness of the mammary epithelial cells to hormones and growth factors and on the activation of various intracellular signaling pathways [51–53].

We observed that Pin1 knockout mice display a developmental defect during pregnancy in preparation for lactation [37]. Before pregnancy, the mammary epithelial ducts from both wild-type and Pin1−/− female mice develop normally. These results indicate that Pin1 is not required for the development of the primary mammary tree, but rather is required for the rapid and timely expansion of mammary epithelial compartment in preparation for lactation. During pregnancy, the mammary epithelial ducts rapidly extend their side branches and build up alveoli, which replace the mammary fat pad and form lobules in preparation for lactation. Pin1−/− female mice, however, show severely reduced mammary epithelial duct development during pregnancy, and the mammary gland fails to undergo the usual massive expansion [37]. This phenotype in the Pin1 knockout mouse is strongly reminiscent of the phenotype resulting from the deletion of cyclin D1, which has a well-established role in the development of breast epithelial cells [54,55]. Consistently, protein levels of cyclin D1 are decreased in mammary epithelial cells of Pin1 knock-out mice [37]. These results indicate that Pin1 plays an important role in breast development, probably via regulation of cyclin D1 function. This conclusion has been independently supported by various studies linking Pin1 to cyclin D1 and breast cancer, as described below.

**Pin1 in breast cancer**

**Overexpression of Pin1 in human breast cancer**

Given that Pin1 regulates the conformation of certain phosphorylated Ser/Thr-Pro motifs – the critical motifs in breast cancer – an interesting question is whether Pin1 expression is aberrantly regulated in human breast cancer. Indeed, striking differences have been found in the levels of expression of Pin1 protein in normal and primary human breast cancer tissues [34]. In this study, Pin1 was overexpressed in 71% of grade II tumors and 90% of grade III tumors. Although there were considerable interindividual variations, especially in grade II and III tumors, the mean expression level of Pin1 in cancer samples was about 10 times that in normal controls. Pin1 levels positively correlate with the tumor grade in invasive breast cancer. In addition, its levels were considerably higher in cell lines derived from human breast cancers than in either normal mammary epithelial cells or cell lines established from such cells [34]. It can be concluded that Pin1 is overexpressed in human breast cancer.

One of the exciting findings from analyzing Pin1 expression in human breast cancer is that levels of this isomerase significantly correlate with cyclin D1 and β-catenin [34,35], two strong and independent prognostic factors for human breast cancer [8,56–58]. In confirmation of previous reports [12,13], in the study cited above cyclin D1 was overexpressed in about 50% of patient samples (24 of the 51) [34]. Of these tumors overexpressing cyclin D1, over 80% also had high levels of Pin1, on average twice as high as in cyclin-D1-negative tumors. Furthermore, there is a link between Pin1 overexpression and cyclin D1 transcription, as all but one patient with high cyclin D1 mRNA levels also had high Pin1 levels, while a few patients had high Pin1 but low cyclin D1 mRNA levels [34].

There is also a strong correlation between Pin1 expression and the level and subcellular localization of β-catenin [35]. Both Pin1 and β-catenin are highly overexpressed in breast cancer tissues in comparison with normal tissues, with the levels of Pin1 significantly correlating with those of β-catenin [35]. Moreover, in tumor tissues with high levels of Pin1, β-catenin accumulates in the nuclear/cytoplasmic fraction, whereas in tumor tissues containing low levels of Pin1, β-catenin is primarily localized at membranes [35]. Given the prognostic value of cyclin D1 levels and the nuclear/cytoplasmic fraction of β-catenin in breast cancer [8,56–58], Pin1 expression is a new potential prognostic marker for breast cancer.
Among other breast cancer markers there is a trend towards a correlation of Pin1 with Neu/ErbB2/Her2 or estrogen receptor negative status. But this did not reach statistical significance, probably because of the small number of estrogen-receptor-negative and/or Her2/Neu-positive patients in the study [34]. It is of interest, however, that the mean Pin1 levels in estrogen-receptor-negative patients and in Her2/Neu-positive patients are, respectively, about twice and 1.7 times those in normal controls [34]. Further studies in larger cohorts may clarify the relation of Pin1 expression to these unfavorable biochemical markers and establish whether Pin1 expression in itself is a useful additional marker for breast cancer prognosis.

Mechanisms of Pin1 overexpression in breast cancer
The causes of elevated Pin1 levels in human breast cancer samples are not fully clear. Given that the cyclin D1 gene is amplified in 10–15% of breast cancers [59], it would be interesting to determine whether Pin1 overexpression is due to any genetic alterations. However, the Pin1 gene is one of those that are most drastically suppressed by up-regulation of BRCA1, as detected in cDNA array screening and Northern analysis [60]. These results suggest that Pin1 levels themselves are tightly regulated, and that high Pin1 levels in cancer may be a result of deregulation at the transcriptional or post-transcriptional level. Indeed, findings in our laboratory indicate that Pin1 is tightly regulated at both the transcriptional and post-transcriptional levels [36,61].

One of the transcriptional factors regulating Pin1 expression is E2F, which binds and activates the PIN1 promoter in vitro and in vivo [36]. Furthermore, the binding of E2F1 to the PIN1 promoter in vivo was strongly correlated with Pin1 expression in breast cancer cell lines [36]. Deregulation of E2F/Rb pathways are observed in breast cancer tissues and cell lines [1,62–66]. Therefore one might speculate that deregulation of E2F plays a role in the up-regulation of Pin1 protein levels in breast cancer.

In addition to transcriptional regulation, Pin1 is regulated by phosphorylation, which fluctuates during the cell cycle [61]. One of the Pin1 phosphorylation sites has been mapped to Ser16 at the center of the shadow pSer/Thr-Pro-binding pocket of the WW domain [61]. Phosphorylation of Ser16 would introduce a negatively charged phosphate into the binding pocket and prevent the WW domain from interacting with pSer/Thr-Pro motifs. Indeed, phosphorylation of Ser16 completely abolishes the ability of Pin1 to interact with its substrates and to carry out its functions, and to clone the protein kinases and phosphatases responsible for modulating its phosphorylation.

Role of Pin1 in the transformation of breast epithelial cells
Given the overexpression of Pin1 in human breast cancer, a critical question is whether this overexpression affects some oncogenic pathways and/or contributes to the transformation of breast epithelial cells. Of the many Pin1 substrates identified so far [3], cyclin D1 is the most extensively studied [34–37] and has a well-established function in breast cancer [11–16]. Consistent with the close correlation between Pin1 and cyclin D1 observed in human breast cancer [34], Pin1 positively regulates cyclin D1 function at the transcriptional level, in collaboration with several different oncogenic signaling pathways and also through post-translational stabilization [34,35,37].

Pin1 regulates expression of cyclin D1 in collaboration with Ras and Wnt/β-catenin signaling pathways. Ras signaling activates the mitogen-activated protein kinase c-Jun N-terminal kinases, which phosphorylate c-Jun on two critical amino terminal Ser-Pro motifs (S63/73-P) thereby enhancing its transcriptional activity [7,14,54,55,67–75]. Pin1 binds c-Jun phosphorylated on Ser 63 and 73, and also increases its ability to activate the cyclin D1 promoter in cooperation with activated c-Jun N-terminal kinase or oncogenic Ha-Ras [34]. Activation of the Wnt/β-catenin signaling pathway is another mechanism leading to cyclin D1 activation and a major feature of breast cancer [8,76–78]. Pin1 binds exclusively to phosphorylated β-catenin close to the APC interaction site. APC (adenomatous polyposis coli [protein]) is a nuclear–cytoplasmic shuttling protein that can export nuclear β-catenin to the cytoplasm for degradation [35]. Pin1 binds and isomerizes the pSer246–Pro peptide bond in β-catenin, which inhibits its ability to bind APC, thereby stabilizing β-catenin and causing its nuclear accumulation [35].

In addition, Pin1 can bind and stabilize cyclin D1 protein [37]. Phosphorylation of cyclin D1 by GSK-3β (glycogen synthase kinase 3 beta) on the Thr286-Pro site regulates turnover and localization of cyclin D1 by enhancing its binding to the nuclear transporter CRM1 and nuclear export, which leads to degradation of cyclin D1 in the nucleus [9,10]. Pin1 binds and stabilizes phosphorylated cyclin D1, presumably by preventing its nuclear export and proteolysis in cytoplasm [37]. Thus, Pin1 positively regulates cyclin D1 function at both transcriptional and post-translational levels, and this may explain why loss of Pin1 function in the mouse mimics the cyclin-D1-null phenotypes [37].

Recent data support the notion that Pin1 can partially transform mammary epithelial cells [36]. Overexpression of Pin1 can confer anchorage-independent cell growth to MCF-10A cells, a spontaneously immortalized, but non-transformed, mammary epithelial cell line [36,79,80]. Furthermore, MCF-10A cells overexpressing Pin1 fail to
undergo normal cell differentiation and acinal formation when cultured in three-dimensional matrigel, with the phenotype consistent with early transformation [36,80]. However, Pin1 overexpression seems not to affect cell growth or cell morphology under normal growth conditions [36]. It is possible that Pin1 becomes oncogenic only after activation of some other oncogenic pathways leading to phosphorylation of its substrates. Indeed, Pin1 greatly enhances and facilitates transformation induced by oncogenic Neu and Ras in mammary epithelial cells [36]. In contrast, inhibition of Pin1 inhibits both cell proliferation and the transformed phenotypes induced by Neu/Ras oncogenes [36]. Importantly, these transformed phenotypes suppressed by inhibition of Pin1 can be fully rescued by overexpression of a constitutively active cyclin D1 mutant that is refractory to the Pin1 inhibition [36]. These results indicate that Pin1 is essential for the Neu/Ras-induced transformation of mammary epithelial cells and that cyclin D1 is the major target of Pin1 during this transformation.

On the basis of these results, we propose that Pin1, whose expression is activated by oncogenic pathways, can cooperate with oncogenic pathways in a 'post-phosphorylational' regulation where the prompt cis–trans isomerization of prolyl residues adjacent to phosphorylated serine or threonine residues promotes cell proliferation and transformation. In this model, Pin1 is an indispensable translator and amplifier of oncogenic signal transduction, which responds to oncogenic signaling and translates it into cell proliferation and transformation.

**Pin1 as a new drug target for breast cancer treatment**

In the past several years, the signal transduction cascades have become promising targets for anticancer therapy, especially breast cancer therapy [81–83]. For example, trastuzumab (Herceptin), the monoclonal antibody against Her2/Neu/ErbB2, is now part of the standard armamentarium in treating metastatic breast cancer, and studies are under way to evaluate its use in adjuvant treatment [84,85]. Flavopiridol is a potent inhibitor of cell division protein kinases (CdkS), especially Cdk4, a key enzyme in the cyclin D1/Rb pathway, and is being evaluated in phase II trials. In preclinical studies, the combination of Flavopiridol and Herceptin has shown synergistic activity in blocking downstream signaling and cyclin D1 activation [86]. ZD1839 (Iressa) is a selective inhibitor of the epidermal growth factor receptor tyrosine kinase, with substantial clinical activity in a number of adenocarcinomas. Interestingly, tumors positive for Her2/neu are especially sensitive to Iressa, and in vitro Iressa and Herceptin have considerable synergistic anticancer activity [87,88]. Finally, the function of Ras can be inhibited by blocking its post-transcriptional farnesylation, and the efficacy of Ras farnesylation inhibitors (such as tipifarnib) is now being evaluated in clinical trials [89].

Because of its overexpression and its pivotal role at the multiple steps during various oncogenic activation pathways in breast cancer [34–37], Pin1 may become an appealing drug target for breast cancer therapy. Inhibition of Pin1 would block activation of cyclin D1 through multiple mechanisms [34–37]. Since the absence of cyclin D1 has been shown to protect mice from breast cancers induced by Ras or Her2/Neu [16], the inhibition of Pin1 might be similarly protective. Furthermore, even partial inhibition of Pin1 appears to be sufficient to suppress the transformed phenotype of breast epithelial cells, while it might not be generally toxic [36]. The idea that Pin1 depletion is conceivably not generally toxic is supported by the findings that suppression of Neu- and Ras-induced transformed phenotypes by Pin1 inhibition can be rescued by overexpression of a constitutively active cyclin D1 mutant that is refractory to the Pin1 inhibition [36].

Although Pin1 inhibition with antisense strategies and dominant-negative mutants has been employed successfully in vitro [17,36,61,90], the feasibility of therapeutic Pin1 inhibition has not yet been explored. An active yet nonspecific PPIase inhibitor is the naturally occurring naphthoquinone derivative juglone [91]. This derivative covalently inactivates a unique cysteine in the active site of the third family of PPIases, including Pin1-type and parvulin-type PPIases [91]. Juglone has been shown to have some anticancer activity and has been used as a Pin1 inhibitor in some studies in cells [90,92,93]. However, since juglone has been shown to inhibit many other proteins and enzymes, even some with a much higher potency than Pin1 [92,94–96], its metabolic effects are clearly not restricted to PPIase inhibition. Therefore, there is a need for the development of highly Pin1-specific inhibitors. The development of such specific inhibitors with a favorable toxicity profile may open up new treatment options for breast cancer. They could be efficacious in themselves or in combination with either classic chemotherapy or other molecularly targeted anticancer drugs aimed at disrupting Her2/Neu or Ras signaling.

**Role of Pin1 in other cell types and cancers**

Various studies suggest that Pin1 might also play an important role in many other cell types and cancers, besides its role in breast development and breast cancer. Following the original identification of the human Pin1 as a protein that is able to interact with and suppress a mitotic kinase [17], subsequent studies have confirmed that Pin1 is a critical mitotic regulator that modulates the function of many mitotically phosphorylated proteins [18,23,25,28–30]. Further studies indicate that Pin1 also plays a critical role in other cell cycle transition points, notably the G1/S transition by modulating some key G1/S regulators.
such as cyclin D1 and its upstream regulators [34–37], as well as cell cycle checkpoint regulation induced by inhibiting DNA synthesis [32] or damaging DNA [45–47].

Consistent with the important roles of Pin1 in the cell cycle, its expression varies greatly among different tissues and cell types in both humans [26,34,35] and mice [37]. Readily detectable levels of Pin1 are generally seen in rapidly proliferating cells such as the urothelium and the epithelial cells located at the bottom of colon crypts [34,37]. Furthermore, loss of Pin1 function in the mouse causes a range of abnormalities due to defective cell proliferation and differentiation. These include decreased body size, testicular atrophy, and retinal degeneration, in addition to defects in breast epithelial cells [37]. However, not all rapidly dividing normal cells or tumors are Pin1-positive [34,35], and some nondividing neurons in humans [26] and mice [37] also express Pin1. Therefore, this isomerase is probably not a general proliferative marker and may have different functions in nondividing and dividing cells [34,35,37]. It is specifically overexpressed in a number of human adenocarcinomas such as colon, prostate, and lung cancer, and in sarcomas such as lymphoma and melanoma [34,35]. In contrast, inhibition of the Pin1 function in multiple human cancer cell lines using expression of a Pin1 antisense RNA or dominant-negative mutants induces mitotic arrest and apoptosis [17,61,90]. These various findings indicate that Pin1 probably plays an important and specific role in regulating cell proliferation under physiological and oncogenic conditions. Further experiments are required to elucidate its biological functions and underlying molecular mechanisms in different cell types and to determine the significance of its overexpression in the development, diagnosis, prognosis, and treatment of various human cancers.

**Conclusion**

Recent studies indicate that phosphorylation-dependent prolyl isomerization is a new post-phosphorylation signaling mechanism that plays an important role in diverse cellular processes. Importantly, this mechanism has provided new insights into breast development and cancer. Pin1 is overexpressed in breast cancer and may function to translate or amplify multiple oncogenic signaling mechanisms during oncogenesis. In contrast, inhibition of Pin1 could provide a unique way of disrupting oncogenic pathways and therefore represent an appealing target for anticancer therapies. A major challenge for the future will be to develop various animal models to further elucidate the molecular mechanisms by which Pin1 regulates breast development and cancer and to define the interactions between Pin1 and other oncogenes or tumor suppressors involved in breast cancer. Another major challenge will be to develop Pin1-specific inhibitors and to evaluate their potential for treating breast cancer.

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**Competing interests**

KPL is a consultant to Pintex Pharmaceuticals, Inc.

**Acknowledgement**

GW and AR contributed equally to this work. We are very grateful to B Neel, L Cantley, T Hunter, J Nevins, C Sherr, P Sicinski, J Brugge and M Yamamoto for constructive discussions and/or suggestions. GW is supported by Mentored Clinical Scientist Award K08CA093655 from the NIH, AR and Y-CL are Leukemia and Lymphoma Society Special Fellow and Canadian Institute of Health Research Fellow, respectively. KPL is a Pew Scholar and a Leukemia and Lymphoma Society Scholar. The studies performed in the authors’ laboratory were supported by NIH grants GM56230, GM58556, and AG17870 to KPL.

**References**

1. Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 2000, 100:57-70.
2. Blume-Jensen P, Hunter T: Oncogenic kinase signalling. Nature 2001, 411:355-365.
3. Lu KP, Liu YC, Zhou ZX: Pinning down the proline-directed phosphorylation signaling. Trends Cell Biol 2002, 12:164-172.
4. Andrechek ER, Muller WJ: Tyrosine kinase signalling in breast cancer: tyrosine kinase-mediated signal transduction in transgenic mouse models of human breast cancer. Breast Cancer Res 2000, 2:211-216.
5. Lee RJ, Albanese C, Fu M, D’Amico M, Lin B, Watanabe G, Haines GK, 3rd, Siegel PM, Hung MC, Yarden Y, Horowitz JM, Muller WJ, Pestell RG: Cyclin D1 is required for transformation by activated Neu and is induced through an E2F-dependent signaling pathway. Mol Cell Biol 2000, 20:672-683.
6. Harari D, Yarden Y: Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. Oncogene 2000, 19:6102-6114.
7. Albanese C, Johnson J, Watanabe G, Eklund N, Vu D, Arnold A, Pestell RG: Transformation by c-ErbB2 mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions. J Biol Chem 1995, 270:23589-23597.
8. Lin SY, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y, Pestell RG, Hung MC: Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proc Natl Acad Sci USA 2000, 97:4262-4266.
9. Diehl JA, Zindy F, Sherr CJ: Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. Genes Dev 1997, 11:957-972.
10. Diehl JA, Cheng M, Roussel MF, Sherr CJ: Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. Genes Dev 1998, 12:3499-3511.
11. Alt JR, Cleveland JL, Hannink M, Diehl JA: Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. Genes Dev 2000, 14:3102-3114.
12. Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, Barnes D, Peters G: Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res 1994, 54:1812-1817.
13. Bartkova J, Lukas J, Muller H, Lutzhoft D, Strauss M, Bartek J: Cyclin D1 protein expression and function in human breast cancer. Int J Cancer 1994, 57:353-361.
14. Hinds PW, Dewsy SF, Eaton EN, Arnold A, Weinberg RA: Function of a human cyclin gene as an oncogene. Proc Natl Acad Sci USA 1994, 91:709-713.
15. Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV: Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature 1994, 369:669-671.
16. Yu Q, Geng Y, Sicinski P: Specific protection against breast cancers by cyclin D1 ablation. Nature 2001, 411:1017-1021.
17. Lu KP, Hanes SD, Hunter T: A human peptidyl-prolyl isomerase essential for regulation of mitosis. Nature 1996, 380:544-547.
18. Yaffe MB, Schutkowski M, Shen M, Zhou ZX, Stukenberg PT, Rahfeld J, Xu J, Kuang J, Kirschner MW, Fischer G, Cantley LC, Lu KP: Sequence-specific and phosphorylation-dependent prolyl isomerization: A potential mitotic regulatory mechanism. Science 1997, 278:1957-1960.
19. Ranganathan R, Lu KP, Hunter T, Noel JP: Structural and functional analysis of the mitotic peptidyl-prolyl isomerase Pin1 suggests that substrate recognition is phosphorylation dependent. Cell 1997, 89:875-886.
Dolinski K, Maur U, Cardenas M, Heitman J: All cyclins and FBPs, individually and collectively, are dispensable for viability in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 1997, 94:13093-13098.

Dolinski K, Stuebenberg PT, Kirschner MW, Lu KP: The essential mitotic peptidyl-prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins. Genes Dev 1998, 12:706-720.

Crenshaw DG, Yang J, Means AR, Kornbluth S: The mitotic peptidyl-prolyl isomerase, Pin1, interacts with Cdc25 and Ptk1. EMBO J 1998, 17:1315-1327.

Lu PJ, Zhou ZX, Shen M, Lu KP: A function of WW domains as phosphoserine- or phosphothreonine-binding modules. Science 1999, 283:1325-1328.

Patra D, Wang SX, Kumagai A, Dunphy WG: Accumulation of rab4GTP in the cytoplasm and association with the peptidyl-prolyl isomerase Pin1 for the replication checkpoint. Mol Cell 2000, 6:873-886.

Stuebenberg PT, Kirschner MW: Pin1 acts catalytically to promote a conformational change in Cdc25. Mol Cell 2001, 7:1071-1083.

Patra D, Wang SX, Kumagai A, Dunphy WG: The xenopus Suc/1/ks1 protein promotes the phosphorylation of G(2)/M regulators. J Biol Chem 1999, 274:38839-38842.

Fujimori F, Takahashi K, Uchida C, Uchida T: The essential prolyl isomerase Pin1 during mitosis. Mol Cell Biol 2000, 11:2201-2211.

Wulf GM, Ryo A, Wulf GG, Lee SW, Liou YC, Lu KP: Pin1 is overexpressed in breast cancer and potentiates the transcriptional activity of the E2F target gene essential for the Neu/Ras-induced transformation of mammary epithelial cells. Mol Cell Biol 2001, 21:3459-3472.

Ryo A, Nakamura N, Wulf G, Liou YC, Lu KP: Pin1 regulates turnover and subcellular localization of beta-catenin by inhibiting its interaction with APC. Nature Cell Biol 2001, 3:763-771.

Ryo A, Liou YC, Wulf G, Nakamura N, Lee SW, Lu KP: Pin1 is an E2F target gene essential for the Neu/Ras-induced transformation of mammary epithelial cells. Mol Cell Biol 2002, 22:5281-5291.

Wu X, Wilcox CB, Devasahayam G, Hackett RL, Arevalo-Rodriguez M, Cardenas M, Heitman J, Harocosteanu S, Takeda Y, Goodwin A: Cyclin H interacts with and regulates silencing by the Sin3-Rpd3 histone deacetylase. EMBO J 2000, 19:3739-3749.

Wu X, Wilcox CB, Devasahayam G, Hackett RL, Arevalo-Rodriguez M, Cardenas M, Heitman J, Harocosteanu S, Takeda Y, Goodwin A: Cyclin H interacts with and regulates silencing by the Sin3-Rpd3 histone deacetylase. EMBO J 2000, 19:3739-3749.
67. Hunter T, Karin M: The regulation of transcription by phosphorylation. Cell 1992, 70:375-387.
68. Whitmarsh AJ, Davis RJ: Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. J Mol Med 1995, 74:589-607.
69. Karin M, Liu Z, Zandi E: AP-1 function and regulation. Curr Opin Cell Biol 1997, 9:240-246.
70. Binetruy B, Smeal T, Karin M: Ha-Ras augments c-Jun activity and stimulates phosphorylation of its activation domain. Nature 1991, 351:122-127.
71. Smeal T, Binetruy B, Mercola DA, Birrer M, Karin M: Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. Nature 1991, 354:405-406.
72. Denjard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, Karin M, Davis RJ: JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell 1994, 76:1025-1037.
73. Rothen G, Denomme GD, Reutens AT, Fu M, Watanabe G, Lee RJ, Kitas RN, Henglein B, Avargradi S, Somasundaram K, Thimmappaya B, Pestell RG: Activation of the cyclin D1 gene by the E1A-associated protein p300 through AP-1 inhibits cellular apoptosis. J Biol Chem 1999, 274:34186-34195.
74. Bakri L, Lallemand D, Bossuyt-Wetzel E, Yaniv MJ: Cell cycle-dependent variations in c-Jun and JunB phosphorylation: a role in the control of cyclin D1-deficient mice. EMBO J 2000, 19:2056-2068.
75. Robles de Rodriguez-Puebla ML, Glick AB, Trembus C, Hansen L, Sicinski P, Tennant RW, Weinberg RA, Yap SH, Conti CJ: Reduced tumor cell development in cyclin D1-deficient mice highlights the oncogenic ras pathway in vivo. Genes Dev 1998, 12:2469-2474.
76. Roose J, Huls G, van Beest M, Moer K, van der Horn K, Goldschmeding R, Logtenberg T, Clevers H: Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1. Science 1999, 285:1923-1926.
77. Jonsson M, Borg A, Nilbert M, Andersson T: Involvement of adenomatous polyposis coli (APC)/beta-catenin signalling in human breast cancer. Eur J Cancer 2000, 36:224-246.
78. Schlosshauer PW, Brown SA, Eisinger K, Yan Q, Guglielminetti J, Parsons R, Ellenson LH, Kitajewski J: APC truncation and increased beta-catenin levels in a human breast cancer cell model. Carcinogenesis 2000, 21:1453-1456.
79. Petersen OW, Ronnov-Jessen L, Howlett AR, Bissell MJ: Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proc Natl Acad Sci USA 1992, 89:9064-9068.
80. Muthuswamy SK, Li D, Lelievre S, Bissell MJ, Brugge JS: ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. Nat Cell Biol 2001, 3:785-792.
81. Druker BJ: Perspectives on the development of a molecularly targeted agent. Cancer Cell 2002, 1:31-36.
82. Chabner BA: Cytotoxic agents in the era of molecular targets and genomics. Oncologist 2002, 7 (suppl 2):34-41.
83. Ghosh S, Liu XP, Zheng Y, Uckun FM: Rational design of potent and selective EGFR tyrosine kinase inhibitors as anticancer agents. Curr Cancer Drug Targets 2001, 1:129-140.
84. Mould SL, Yakes FM, Muthuswamy SK, Bianco R, Simpson JF, Arteaga CL: Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells in vitro and in vivo. Cancer Res 2001, 61:8887-8895.
85. Arteaga CL, Mould SL, Yakes FM: HER (erbB) tyrosine kinase inhibitors in the treatment of breast cancer. Semin Oncol 2002, 29:4-10.
86. Nahta R, Iglesiat JD, Kempkes B, Schmidt EV: Rate-limiting effects of Cyclin D1 in transformation by ErbB2 predicts synergy between herceptin and flavopiridol. Cancer Res 2002, 62:2267-2271.
87. Moasser MM, Basso A, Averbuch SD, Rosen N: The tyrosine kinase inhibitor ZD1839 (‘Iressa’) inhibits HER2-driven signal transduction and suppresses the growth of HER2-overexpressing tumor cells. Cancer Res 2001, 61:7184-7188.
88. Albanell J, Codony-Servat J, Rojo F, Del Campo JM, Saulea S, Anido J, Raspail G, Giralt J, Roselló J, Nicholson R, Mendelsohn J, Baselga J: Activated extracellular signal-regulated kinases: association with epidermal growth factor receptor/transforming growth factor alpha expression in head and neck squamous carcinoma and inhibition by anti-epidermal growth factor receptor treatments. Cancer Res 2001, 61:6500-6510.
89. Karp JE, Kaufmann SH, Adjei AA, Lancet JE, Wright JI, End DW: Current status of clinical trials of farnesyltransferase inhibitors. Curr Opin Oncol 2001, 13:470-476.
90. Rippmann JF, Hobbie D, Saiber C, Guillard B, Bauer M, Birk J, Derijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, Karin M, Davis RJ: Synergy between Herceptin and Flavopiridol. Cancer Res 2000, 11:409-416.
91. Hennig L, Christner C, Kipping M, Schellbert B, Rucknagel KP, Grabley S, Kullert G, Fischer G: Selective inactivation of parvin-like peptide-PROL-cis/trans isomerases by juglone. Biochemistry 1998, 37:5953-5960.
92. Chao SH, Greenleaf AL, Price DH: Juglone, an inhibitor of the peptide-prolyl isomerase Pin1, also directly blocks transcription. Nucleic Acids Res 2001, 29:767-773.
93. He J, Lau AG, Yaffe MB, Hall RA: Phosphorylation and cell cycle-dependent regulation of the Na+/H+ exchanger regulatory factor (NHERF-1) by cdc2 kinase. J Biol Chem 2001, 276:41569-41575.
94. Muto N, Inouye K, Inada A, Nakaniishi T, Tan L: Inhibition of cytochrome P-450-linked monooxygenase systems by naphthoquinones. Biochem Biophys Res Commun 1987, 146:487-494.
95. Duhamain AS: Characterization of zeta-crystallin inhibition by juglone. Biochem Biophys Res Commun 1998, 218:648-652.
96. Munday R, Munday CM: Induction of quinone reductase and glutathione transferase in rat tissues by juglone and plumagin. Planta Med 2000, 66:399-402.