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RESEARCH

# Prolyl Isomerase Pin1 Regulated Signaling Pathway Revealed by Pin1 $^{+/+}$ and Pin1 $^{-/-}$ Mouse Embryonic Fibroblast Cells

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**Abstract** Pin1 (peptidylprolyl cis/trans isomerase, NIMA-interacting 1) plays a key role in a number of diseases including cancer and Alzheimer disease. Previous studies have identified a wide range of phosphoproteins as Pin1 substrates. Related pathways were analyzed separately. The aim of this study was to provide a comprehensive picture involving Pin1 regulation. A genome-wide mRNA expression microarray was carried out using the RNA isolation from Pin1 $^{+/+}$ and Pin1 $^{-/-}$ mouse embryonic fibroblast (MEF) cells. Signaling pathways regulated by Pin1 were analyzed with the utility of KEGG pathway and GO annotation. An expression pattern regulated by Pin1 was revealed. A total of 606 genes, 375 being up-regulated and 231 down-regulated, were differentially expressed when comparing Pin1 +/+ to Pin1 −/− MEF cells. Totally 48 pathways were shown to be regulated by Pin1 expression in KEGG pathway analysis. In the GO annotation system, 19 processes on biological processes, 15 processes on cellular components, and 18 processes on molecular functions were found to be in the regulation of Pin1 expression. Pathways related to immune system and cancer showed most significant association with Pin1 regulation. Pin1 is an important regulator in a wide range of signaling pathways that were related to immune system and cancer.

**Keywords** Pin1 · Pathway · Cancer · Immune

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## Introduction

Peptidyl prolyl isomerase Pin1 (peptidylprolyl cis/trans isomerase, NIMA-interacting 1) was first discovered in a molecular screen for mitosis regulation, belonging to the PPIase-parvulin family [1]. Pin1 has two domains: a WW domain that binds to the phospho-Ser/Thr-Pro motifs, and a distinctive PPIase catalytic domain [2, 3]. As a highly conserved enzyme, Pin1 isomerizes specific phosphorylated Ser/Thr-Pro bonds, induces conformational changes of a defined subset of phosphoproteins and thereby regulating protein function [4]. A number of proteins are found to be Pin1 substrates, including Cyclin D1, β-Catenin, p53, c-Jun, Nanog, c-Fos, Bcl-2 and so on [5–12], involved in the regulation of several major cellular signaling pathways [13]. Pin1 plays a key role in a number of diseases, notably ageing and age-related diseases, including cancer and Alzheimer disease [14]. By presenting a novel mechanism in cell signaling regulation, Pin1 represents a potentially novel molecular marker for tumor diagnosis or prognosis, and molecular target for cancer therapy [15].

However, studies on Pin1 mainly focused on only one substrate or one pathway that was regulated by Pin1. The network connecting between Pin1 substrates and downstream molecules, or related pathways was seldom analyzed. Since multiple phosphoproteins were recognized as the substrates of Pin1, it seemed to be biased when analyzing only one substrate. The effect of Pin1 on cellular process should be considered synergistically. Pin1 $^{+/+}$ and Pin1 $^{-/-}$ mouse embryonic fibroblast (MEF) cells were derived from mouse embryos of the wild-type (Pin1$^{+/+}$) and the mutant form (Pin1$^{-/-}$) separately. Pin1 $^{-/-}$ MEF cells grow slower than Pin1 $^{+/+}$ ones, and are defective in entering cell cycle from

$G_0$ arrest [16]. In this study, we performed a genome-wide mRNA expression profile revealing the difference between Pin1 $^{+/+}$ and Pin1 $^{-/-}$ MEF cells. More importantly, pathways regulated by Pin1 were analyzed using KEGG pathway and GO annotation in order to provide a comprehensive picture involving Pin1 regulation. Thus the Pin1 regulated molecular mechanisms were further revealed in a whole view.

## Materials and Methods

### Cell Culture

Pin1 $^{+/+}$ and Pin1 $^{-/-}$ mouse embryonic fibroblast (MEF) cells were kindly provided by Professor Dong (The Hormel Institute, University of Minnesota) and originally generated by Fujimori et.al. [16]. The cells were cultured in DMEM with 10 % fetal bovine serum at 37 °C in a humidified chamber containing 5 % $CO_2$. Cells were seeded into 60 mm culture dishes ($5\times10^5$ per dish) for 2 days before RNA isolation.

### RNA Isolation

Total RNA was extracted from cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. For the microarray studies, the quality and concentration of the RNA samples were assessed by NanoDrop ND-1000 (Thermo Fisher Scientific Inc) and RNA electrophoresis. Qualified RNA samples with A260/A280 ratio of 1.8–2.0 and 28S/18S rRNA ratio greater than 1:1 were used for microarray analysis.

### Microarray Experiments on CapitalBio Platform

Microarray studies were carried out by CapitalBio Corporation (Beijing, China). The mouse genome-wide 70-mer microarray was fabricated in-house at CapitalBio [17, 18]. Briefly, a mouse genome oligonucleotide set (version 4.0, from Operon.) consisting of 5′ amino acid-modified 70-mer probes and representing 32,256 Mus musculus genes, and internal and external controls, were printed on PolymerSlide, in which the surface was covered by a thin layer of aldehyde group modified three-dimensional polymer chain (CapitalBio Corp.).

cDNA was labeled with a fluorescent dye (Cy5 and Cy3-dCTP) according to Eberwine's linear RNA amplification method and subsequent enzymatic reaction [18]. Improvements for producing higher yields of labeled cDNA have been made by using CapitalBio cRNA Amplification and Labeling Kit (CapitalBio). Labeled cDNA was purified with a PCR NucleoSpin Extract II Kit (MN) and resuspended in elution buffer. Labeled DNA (labeled controls and test samples) was dissolved in 80 μL hybridization solution (3× SSC, 0.2 % SDS, 5×Denhardt's solution and 25 % formamide), and then denatured at 95 °C for 3 min prior to loading onto a microarray. Array was hybridized at 42 °C overnight in a CapitalBio BioMixer$^{TM}$ II Hybridization Station with a rotation speed of 8 rpm and washed with two consecutive solutions (0.2 % SDS, 2× SSC at 42 °C for 5 min, and 0.2× SSC for 5 min at room temperature) before scanning with a confocal LuxScan$^{TM}$ scanner (CapitalBio). The scanning setting for the Cy3 and Cy5 channels of two-color microarrays was manually balanced by visual inspection of the external control spots. The images obtained were then analyzed using LuxScan$^{TM}$ 3.0 software (CapitalBio).

### Data Processing and Pathway Analysis

The scanned signals were extracted with LuxScan$^{TM}$ 3.0 software (CapitalBio). Gene was recognized as differentially expression when fold change of signal was greater than 2. The dataset consisting of the differentially expressed genes was entered into Molecule Annotation System 3.0 (MAS from CapitalBio, http://bioinfo.capitalbio.com/mas3/), a website based program that is designed for microarray data analysis and molecule annotation. MAS uses the annotations from the KEGG and GO Consortium to identify global biological trends in gene expression data. MAS calculates the total number of genes changed within a KEGG pathway or a GO term, and a statistical score ($p$-value and $q$-value, or false discovery rate), providing a comprehensive view of the gene expression differentiation associated with a particular KEGG pathway or GO term.

### Quantitative Real-Time PCR

Eight candidate genes were selected for real-time PCR validation. cDNAs were prepared from 2 μg of total RNA using MMLV reverse transcription kit according to the manufacturer's protocol (Promega, USA). qPCR was performed with FS Universal SYBR Green Master reagents (Roche, USA) in an Applied Biosystems PRISM 7500 instruments according to the manufacture's protocol. The reaction mix had a total volume of 15 μl containing 1×SYBR Green Master Mix, 5 pmol of forward and reverse primers, and 0.5 μl of cDNA. The reactions were incubated in a 96-well plate at 95 °C for 10 min, followed by 45 cycles of 95 °C for 30 s and 60 °C for 1 min. All reactions were run in duplicate. The primers for the eight candidate genes and the house keeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were listed in Table 1. The comparative Ct method (ΔΔCt) was used for quantification of gene expression and relative quantification (RQ) was calculated as $2^{-\Delta\Delta Ct}$.

**Table 1** Primer pairs used for quantitative RT-PCR

| Gene | Reference sequence | Forward primer | Reverse primer |
|---|---|---|---|
| Serpina3n | NM_009252.2 | CAGTGCCATGTTCATCCTCCCT | AGCTCATCTATCATCCTGGGTTTCA |
| Lcn2 | NM_008491.1 | TCAAAATTACCCTGTATGGAAGAACC | TTGTCAATGCATTGGTCGGTG |
| Il1rn | NM_031167.5 | ACTAGAAGAAAAGATAGACATGGTGCC | TCAGATCAGTGATGTTAACTTCCTCC |
| Ppbp | NM_023785.2 | TGGAAAATCTGATGGCATGGAC | TCCATTCTTCAGTGTGGCTATCACT |
| Hspb1(Hsp27) | NM_013560.2 | GGAGCTCACAGTGAAGACCAAGG | ACCTGGAGGGAGCGTGTATTTC |
| Crabp1 | NM_013496.2 | CAAATGCAGGAGTTTACCCACG | GCCAAATGTCAGGATTAGCTCATC |
| Lpl | NM_008509.2 | AGACTCAGAAAAAGGTCATCTTCTGTG | TGTTTGTCCAGTGTCAGCCAGAC |
| Syce2 | NM_001168246.1 | AATTTACCCAGAAGATGGCAAAGATC | GCAAGTGGGAACCTCAGACTGG |
| GAPDH | NM_008084.2 | ATGGCCTTCCGTGTTCCTACC | TTGATGTCATCATACTTGGCAGGTT |

## Results

### Expression Pattern

Genes with more than two fold change were thought to be differentially regulated. Results showed that 606 genes (data not shown), 375 being up-regulated and 231 down-regulated, were differentially expressed when comparing Pin1 $^{+/+}$ to Pin1 $^{-/-}$ MEF cells. Genes with fold change greater than ten were selected to draw an expression pattern between Pin1 $^{+/+}$ and Pin1 $^{-/-}$ MEF cells (Fig. 1). Fifty genes exhibited a ten plus fold change, with 39 genes up-regulated and 11 down-regulated. Genes Serpina3n, Lcn2 and Il1rn showed the greatest up-regulation, and genes Crabp1, Lpl and Syce2 showed the greatest down-regulation.

### KEGG Pathway

KEGG pathway analysis was to illustrate all the available pathways containing differentially expressed genes. We defined the significant regulated pathway with both *p*-value and *q*-value less than 0.001. Totally 48 pathways were shown to be regulated by Pin1 expression (Table 2). The ten most significantly change pathways were antigen processing and presentation, type I diabetes mellitus, allograft rejection, graft-versus-host disease, cytokine-cytokine receptor interaction, systemic lupus erythematosus, cell adhesion molecules (CAMs), autoimmune thyroid disease, MAPK signaling pathway, and focal adhesion.

### GO Annotation

To exhibit a comprehensive picture of global gene expression, we determined groups of functionally relevant genes according to GO annotation. According to the ordering of this ontology system, three main classes of processes were distinguished: (1) biological processes, (2) cellular components, and (3) molecular functions. We defined the significant regulated process with both *p*-value and *q*-value less than 0.001.

On biological processes, there are 19 processes shown to be regulated by Pin1 expression (Table 3). The five most significant processes including: GO:0019882 antigen processing and presentation, GO:0006955 immune response, GO:0002474 antigen processing and presentation of peptide antigen via MHC class I, GO:0010811 positive regulation of

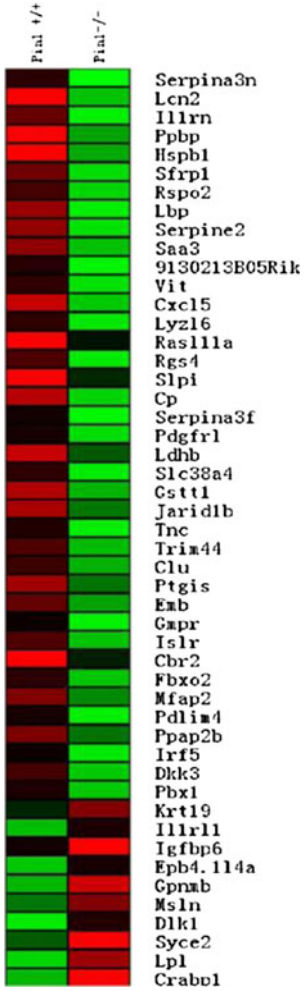


**Fig. 1** Heatmap showed the expression pattern between Pin1 +/+ and Pin1 −/− MEF cells. Genes with fold change greater than 10 were listed

**Table 2** KEGG pathway with significant difference between Pin1 +/+ and Pin1 −/− MEF

| Pathway | Count | *p*-value | *q*-value |
|---|---|---|---|
| Antigen processing and presentation | 15 | 3.91E-17 | 7.08E-15 |
| Type I diabetes mellitus | 13 | 3.74E-16 | 3.38E-14 |
| Allograft rejection | 12 | 5.66E-15 | 2.93E-13 |
| Graft-versus-host disease | 12 | 5.66E-15 | 2.93E-13 |
| Cytokine-cytokine receptor interaction | 18 | 1.69E-14 | 5.43E-13 |
| Systemic lupus erythematosus | 16 | 1.80E-14 | 5.43E-13 |
| Cell adhesion molecules (CAMs) | 15 | 4.88E-14 | 1.26E-12 |
| Autoimmune thyroid disease | 12 | 7.51E-14 | 1.70E-12 |
| MAPK signaling pathway | 16 | 6.79E-12 | 1.37E-10 |
| Focal adhesion | 13 | 1.97E-10 | 3.56E-09 |
| Ether lipid metabolism | 7 | 7.08E-10 | 1.17E-08 |
| Glycerolipid metabolism | 7 | 5.39E-09 | 8.14E-08 |
| Regulation of actin cytoskeleton | 12 | 6.31E-09 | 8.79E-08 |
| Cysteine metabolism | 5 | 8.71E-09 | 1.13E-07 |
| Insulin signaling pathway | 10 | 1.02E-08 | 1.23E-07 |
| Glycerophospholipid metabolism | 7 | 4.90E-08 | 5.54E-07 |
| Axon guidance | 9 | 9.42E-08 | 1.00E-06 |
| TGF-beta signaling pathway | 8 | 1.08E-07 | 1.08E-06 |
| Chronic myeloid leukemia | 7 | 6.21E-07 | 5.91E-06 |
| Glutathione metabolism | 6 | 6.65E-07 | 6.01E-06 |
| Cell cycle | 8 | 7.90E-07 | 6.81E-06 |
| Prostate cancer | 7 | 1.36E-06 | 1.12E-05 |
| Melanoma | 6 | 5.01E-06 | 3.86E-05 |
| p53 signaling pathway | 6 | 5.01E-06 | 3.86E-05 |
| Complement and coagulation cascades | 6 | 5.42E-06 | 3.92E-05 |
| Adherens junction | 6 | 6.33E-06 | 4.41E-05 |
| Glycine, serine and threonine metabolism | 5 | 7.08E-06 | 4.75E-05 |
| Galactose metabolism | 4 | 1.16E-05 | 7.53E-05 |
| Natural killer cell mediated cytotoxicity | 7 | 1.29E-05 | 8.03E-05 |
| Pentose phosphate pathway | 4 | 1.90E-05 | 1.14E-04 |
| Apoptosis | 6 | 2.03E-05 | 1.17E-04 |
| Colorectal cancer | 6 | 2.03E-05 | 1.17E-04 |
| Gap junction | 6 | 2.29E-05 | 1.26E-04 |
| Toll-like receptor signaling pathway | 6 | 3.24E-05 | 1.73E-04 |
| Glioma | 5 | 5.40E-05 | 2.79E-04 |
| Arachidonic acid metabolism | 5 | 6.65E-05 | 3.24E-04 |
| Leukocyte transendothelial migration | 6 | 6.72E-05 | 3.24E-04 |
| Sphingolipid metabolism | 4 | 6.81E-05 | 3.24E-04 |
| VEGF signaling pathway | 5 | 8.11E-05 | 3.76E-04 |
| Tyrosine metabolism | 4 | 8.40E-05 | 3.80E-04 |
| Fructose and mannose metabolism | 4 | 1.02E-04 | 4.52E-04 |
| Fc epsilon RI signaling pathway | 5 | 1.11E-04 | 4.77E-04 |
| Bladder cancer | 4 | 1.24E-04 | 5.21E-04 |
| Tight junction | 6 | 1.69E-04 | 6.96E-04 |
| Ubiquitin mediated proteolysis | 6 | 1.76E-04 | 7.08E-04 |
| Small cell lung cancer | 5 | 2.51E-04 | 9.71E-04 |
| Phenylalanine metabolism | 3 | 2.52E-04 | 9.71E-04 |
| Endometrial cancer | 4 | 2.60E-04 | 9.82E-04 |

**Table 3** Biological process by GO with significant difference between Pin1 +/+ and Pin1 −/− MEF

| GO term | Count | *p*-value | *q*-value |
|---|---|---|---|
| GO:0019882 antigen processing and presentation | 36 | 2.93E-25 | 1.23E-22 |
| GO:0006955 immune response | 53 | 3.81E-19 | 8.57E-17 |
| GO:0002474 antigen processing and presentation of peptide antigen via MHC class I | 16 | 4.94E-16 | 9.53E-14 |
| GO:0010811 positive regulation of cell-substrate adhesion | 13 | 1.03E-13 | 1.55E-11 |
| GO:0001916 positive regulation of T cell mediated cytotoxicity | 12 | 5.99E-13 | 8.09E-11 |
| GO:0042590 antigen processing and presentation of exogenous peptide antigen via MHC class I | 9 | 4.54E-11 | 4.38E-09 |
| GO:0001569 patterning of blood vessels | 10 | 1.29E-10 | 1.08E-08 |
| GO:0030198 extracellular matrix organization and biogenesis | 14 | 1.66E-08 | 1.18E-06 |
| GO:0055114 oxidation reduction | 30 | 1.78E-08 | 1.20E-06 |
| GO:0048538 thymus development | 8 | 2.47E-08 | 1.52E-06 |
| GO:0006364 rRNA processing | 10 | 1.58E-07 | 7.90E-06 |
| GO:0001666 response to hypoxia | 11 | 3.03E-07 | 1.41E-05 |
| GO:0030335 positive regulation of cell migration | 9 | 3.69E-07 | 1.66E-05 |
| GO:0006953 acute-phase response | 7 | 4.06E-07 | 1.74E-05 |
| GO:0006098 pentose-phosphate shunt | 5 | 1.14E-06 | 4.40E-05 |
| GO:0007411 axon guidance | 13 | 2.86E-06 | 1.02E-04 |
| GO:0043627 response to estrogen stimulus | 7 | 1.00E-05 | 3.07E-04 |
| GO:0042036 negative regulation of cytokine biosynthesis | 5 | 1.36E-05 | 3.92E-04 |
| GO:0045454 cell redox homeostasis | 8 | 2.63E-05 | 7.25E-04 |

cell-substrate adhesion, and GO:0001916 positive regulation of T cell mediated cytotoxicity

On cellular components, totally 15 processes were shown to be regulated by Pin1 expression (Table 4). The five most significant processes including: GO:0042612 MHC class I protein complex, GO:0005634 nucleus, GO:0005576 extracellular region, GO:0005737 cytoplasm, and GO:0005783 endoplasmic reticulum.

On molecular functions, 18 processes were shown to be regulated by Pin1 expression (Table 5). The five most significant processes including: GO:0005515 protein binding, GO:0008201 heparin binding, GO:0042605 peptide antigen

**Table 4** Cellular component by GO with significant difference between Pin1 +/+ and Pin1 −/− MEF

| GO term | Count | *p*-value | *q*-value |
|---|---|---|---|
| GO:0042612 MHC class I protein complex | 36 | 1.94E-37 | 2.62E-34 |
| GO:0005634 nucleus | 194 | 3.64E-25 | 1.23E-22 |
| GO:0005576 extracellular region | 97 | 1.42E-23 | 3.82E-21 |
| GO:0005737 cytoplasm | 198 | 2.85E-12 | 3.21E-10 |
| GO:0005783 endoplasmic reticulum | 45 | 8.31E-11 | 7.48E-09 |
| GO:0005615 extracellular space | 32 | 1.56E-10 | 1.24E-08 |
| GO:0005614 interstitial matrix | 8 | 2.36E-09 | 1.77E-07 |
| GO:0016021 integral to membrane | 153 | 2.00E-08 | 1.29E-06 |
| GO:0005739 mitochondrion | 48 | 6.09E-08 | 3.33E-06 |
| GO:0005788 endoplasmic reticulum lumen | 10 | 6.17E-08 | 3.33E-06 |
| GO:0009897 external side of plasma membrane | 21 | 1.07E-07 | 5.53E-06 |
| GO:0005667 transcription factor complex | 20 | 2.32E-06 | 8.45E-05 |
| GO:0001527 microfibril | 4 | 8.62E-06 | 2.74E-04 |
| GO:0000796 condensin complex | 3 | 2.98E-05 | 7.96E-04 |
| GO:0005794 Golgi apparatus | 30 | 3.47E-05 | 9.01E-04 |

**Table 5** Molecular function by GO with significant difference between Pin1 +/+ and Pin1 −/− MEF

| GO term | Count | *p*-value | *q*-value |
|---|---|---|---|
| GO:0005515 protein binding | 265 | 1.11E-26 | 7.49E-24 |
| GO:0008201 heparin binding | 17 | 5.13E-14 | 8.66E-12 |
| GO:0042605 peptide antigen binding | 12 | 2.65E-12 | 3.21E-10 |
| GO:0005509 calcium ion binding | 51 | 3.46E-12 | 3.60E-10 |
| GO:0004867 serine-type endopeptidase inhibitor activity | 14 | 2.86E-08 | 1.68E-06 |
| GO:0004586 ornithine decarboxylase activity | 4 | 2.18E-07 | 1.05E-05 |
| GO:0005520 insulin-like growth factor binding | 7 | 4.06E-07 | 1.74E-05 |
| GO:0050840 extracellular matrix binding | 7 | 5.05E-07 | 2.07E-05 |
| GO:0008009 chemokine activity | 8 | 5.96E-07 | 2.37E-05 |
| GO:0016491 oxidoreductase activity | 37 | 1.61E-06 | 6.04E-05 |
| GO:0003924 GTPase activity | 13 | 3.86E-06 | 1.34E-04 |
| GO:0035014 phosphoinositide 3-kinase regulator activity | 4 | 5.23E-06 | 1.74E-04 |
| GO:0017091 AU-specific RNA binding | 4 | 5.23E-06 | 1.74E-04 |
| GO:0005021 vascular endothelial growth factor receptor activity | 4 | 8.62E-06 | 2.74E-04 |
| GO:0005524 ATP binding | 57 | 1.16E-05 | 3.48E-04 |
| GO:0005525 GTP binding | 21 | 1.30E-05 | 3.81E-04 |
| GO:0008270 zinc ion binding | 72 | 2.07E-05 | 5.83E-04 |
| GO:0004322 ferroxidase activity | 3 | 2.98E-05 | 7.96E-04 |

binding, GO:0005509 calcium ion binding, and GO:0004867 serine-type endopeptidase inhibitor activity

Cancer Related Pathway

Pin1 is an important gene in cancer development and progression. When we focused on the cancer related pathway in KEGG system, we found Pin1 regulated pathway including (Table 2): chronic myeloid leukemia, prostate cancer, melanoma, colorectal cancer, glioma, leukocyte transendothelial migration, bladder cancer, small cell lung cancer, endometrial cancer, cell adhesion molecules (CAMs), MAPK signaling pathway, focal adhesion, TGF-beta signaling pathway, cell cycle, p53 signaling pathway, apoptosis, and VEGF signaling pathway. These pathways regulated cell survival, cell cycle, apoptosis, invasion, and migration during cancer progression.

*RT-PCR Validation*

To determine the reliability of the microarray data, eight differentially expressed genes (Serpina3n, Lcn2, Il1rn, Ppbp, Hspb1, Crabp1, Lpl, and Syce2) with more than twofold changes according to the microarray analysis, were chosen for further confirmation by quantitative RT-PCR. The RT-PCR results of these eight genes agreed well with the microarray (Fig. 2). The results of quantitative RT-PCR were more sensitive than the microarray. The fold changes of RT-PCR were several times greater than microarray data for five genes (Lcn2, Hspb1, Crabp1, Lpl, and Syce2). Overall, these results confirmed our findings of differential gene expression by microarray.

## Discussion

Previous studies have identified a wide range of phosphoproteins as Pin1 substrates. Related pathways were analyzed separately. However, by acting on multiple targets, the effect of Pin1 on cellular process should be considered synergistically. In the present study, the mRNA expression pattern between Pin1 $^{+/+}$ and Pin1 $^{-/-}$ MEF cells was identified. Pin1 regulated pathways were analyzed using KEGG pathway and GO annotation, providing a comprehensive view involving Pin1 regulation.

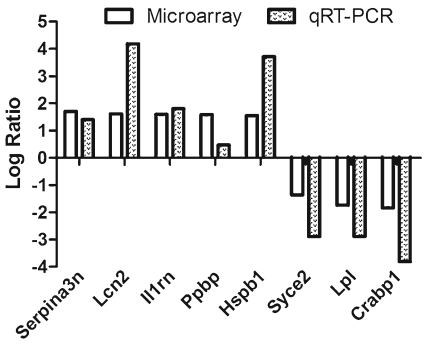


**Fig. 2** Quantitative real-time PCR confirmation of the microarray results. Gene expression levels were normalized to the expression of the house keeping gene GAPDH. Fold change was shown as log ratio between expression of Pin1 +/+ and Pin1 −/−

The greatest up-regulation between Pin1 $^{+/+}$ and Pin1 $^{-/-}$ MEF cells came from genes Serpina3n, Lcn2 and Il1rn, and genes Crabp1, Lpl and Syce2 showed the greatest down-regulation. Most of these genes behave accordantly with Pin1 in immune system or cancer. Serpina3n is a murine serine protease inhibitor. By inhibiting the proapoptotic serine protease Granzyme B mediated decorin degradation, Serpina3n reduces the overall rate of rupture and death in a mouse model of abdominal aortic aneurysm [19]. Lipocalin 2 (Lcn2) is overexpressed in a variety of cancers and promotes survival, growth, and metastasis [20]. Both Serpina3n and Lcn2 function as tumor promotors. The two genes were both up-regulated in Pin1 $^{+/+}$ MEF cells, which indicated the two genes may be involved in Pin1 related cancers. IL-1 receptor antagonist (Il1rn, or IL-1Ra), binding to IL-1 receptors by competing with IL-1 alpha and IL-1 beta, modulates a variety of anti-inflammatory and immune responses [21]. The up-regulation of Il1rn in Pin1 $^{+/+}$ MEF cells suggested the immune function of Pin1 could also be mediated by Il1rn .

The greatest down-regulated genes were Crabp1, Lpl and Syce2. Cellular retinoic acid binding protein 1 (Crabp1) have a tumor-suppressor function in esophageal carcinoma [22]. Lipoprotein lipase (Lpl) gene deficiency increases cancer risk especially in the prostate [23]. Synaptonemal complex central element protein 2 (SYCE2) is necessary to synaptonemal complex assembly, double strand break repair, and thus to complete recombination and meiosis [24]. Among the three genes, Crabp1 and Lpl were both found to have a tumor-suppressor function, and no direct connection between Syce2 and cancer was found. It seemed that Crabp1 and Lpl could mediate the Pin1-promoted cancer.

In the view of pathway analysis, Pin1 regulation showed most significant association with immune system including KEGG pathways, such as antigen processing and presentation, allograft rejection, graft-versus-host disease, cytokine-cytokine receptor interaction, systemic lupus erythematosus, autoimmune thyroid disease, Toll-like receptor signaling pathway, and GO terms GO:0019882 antigen processing and presentation, GO:0006955 immune response, GO:0002474 antigen processing and presentation of peptide antigen via MHC class I, GO:0001916 positive regulation of T cell mediated cytotoxicity, GO:0042612 MHC class I protein complex. Esnault et al. summarized the important role of Pin1 in the immune system, pointing out the modulation by Pin1 of cytokine expression by activated T cells and eosinophils, and participation of Pin1 in T-cell and eosinophil apoptotic decisions both in vitro and in vivo [25]. Interleukin-2 (IL-2), as a critical signaling cytokine in the mammalian immune response, is regulated by at least four families of transcriptional activators: NF-AT, NF-B, Oct, and AP1 (c-Jun and c-Fos), many of which interact with and are regulated by Pin1 [25]. Pin1 blockade greatly attenuates IFN-gamma, IL-2 and CXCL-10 mRNA stability, accumulation and protein expression in activated T cells, regulating the type 1 immune response [26]. In activated T cells, Pin1 is activated by Toll-like receptors TLR7 and TLR9, and then activates IRAK1 with the result of its release from the receptor complex to activate the transcription factor IRF7 and induce type I interferons [27]. The view of pathway analysis in this study provided new insight into the mechanism of how Pin1 participated in immune system.

Because Pin1 plays an important role in cancer, additional analysis was performed for the cancer related pathway regulated by Pin1 in this study. In the KEGG system, Pin1 was associated with several specific cancer pathways directly, such as chronic myeloid leukemia, prostate cancer, melanoma, colorectal cancer, glioma, bladder cancer, small cell lung cancer, and endometrial cancer. Most types of cancer mentioned above were reported to be asscociated with Pin1 except bladder cancer. Pin1 was found to be overexpressed in hematological malignancies (chronic myeloid leukemia) [28], prostate cancer [29, 30], melanoma [31], colorectal cancer [32, 33], glioblastoma [34], non-small cell lung cancer [35, 36], and endometrial carcinoma with morules [37]. The results of this study emphasized the important role of Pin1 in those tumor types and suggested a key role of Pin1 in bladder cancer which was previously uncharacterized.

Pin1 regulates several pathways related to cancer development, including cell adhesion molecules (CAMs), MAPK signaling pathway, focal adhesion, TGF-beta signaling pathway, cell cycle, p53 signaling pathway, apoptosis, and VEGF signaling pathway. It should be acknowledged that pathways of cell cycle and apoptosis control cancer cell survival, while pathways of cell adhesion molecules (CAMs) and focal adhesion modulate cancer cell invasion or migration. MAPK signaling pathway is crucial in numerous stimulated cellular processes, such as proliferation, differentiation, apoptosis and stress response. Dysregulation of this pathway is involved in the induction and progression of cancer [38]. Pin1 is reported to regulate BPGAP1 (a multidomain Rho GTPase-activating protein) function in Rho and Erk signalling [39]. Transforming growth factor-beta (TGF-beta) regulates a wide variety of cellular processes, including proliferation, differentiation, migration, and apoptosis. Pin1 down-regulates Smad2/3 protein levels and therefore negatively regulates TGF-beta signaling [40]. Pin1 is a regulator of p53 in cellular response to genotoxic stress [7, 8]. Pin1 increases VEGF expression level by activating HIF-1alpha and AP-1 in breast cancer cells [41]. The pathway analysis in this study emphasized the important role of Pin1 in those cancer related pathways.

In summary, we determined an expression pattern regulated by peptidyl-prolyl cis-trans isomerase Pin1. Signaling pathways were further analyzed. A variety of pathways have been reported to be associated with Pin1 previously, but a comprehensive view was not showed before. The function

of Pin1 in immune system and cancer was further addressed. Pin1 regulation in bladder cancer was suggested in our pathway analysis. These data also provided a basis for further investigation of Pin1 molecular mechanisms.

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