Differential Gene Expression Profiles of *PPP2R5C*-siRNA-Treated Malignant T Cells

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Recently, alterations in the expression pattern of *PPP2R5C* associated with malignant transformation have been characterized, and *PPP2R5C* overexpression was demonstrated in leukemias. To confirm the role of *PPP2R5C* in proliferation and its molecular mechanism, three *PPP2R5C*-siRNAs and a scrambled nonsilencing siRNA control were used to treat Molt-4 and Jurkat T cells. After nucleofection, *PPP2R5C* expression and biological consequences based on a highly efficient and specific *PPP2R5C*-siRNA were demonstrated by qRT-PCR, CCK-8 assay, Annexin V/PI, and flow cytometry. The global gene expression profile of *PPP2R5C*-siRNA-treated Jurkat T cells was established. A significant reduction in the *PPP2R5C* mRNA level was observed at 24 to 72 h in Molt-4 and Jurkat T cells with all of the *PPP2R5C*-siRNAs. The proliferation rate of Molt-4 and Jurkat T cells transfected with different *PPP2R5C*-siRNAs was significantly decreased at 72 h compared with the control ($p < 0.05$). However, the transfected cells did not show a significant increase in Annexin V/PI-positive cells (apoptosis). The highly efficient *PPP2R5C*-siRNA2 was used to treat Jurkat T cells for gene expression profile analysis. In total, 439 genes were upregulated, and 524 genes were downregulated at least twofold in *PPP2R5C*-siRNA-treated Jurkat T cells. Changes in signaling pathway genes closely related to the TCR, Wnt, calcium, MAPK, and p53 signaling pathways were observed. In conclusion, the suppression of *PPP2R5C* by RNA interference could effectively inhibit the proliferation of leukemic T cells, the *PPP2R5C*-siRNA treatment altered gene expression profiles, and the differential expression of the glycogen synthase kinase 3 beta (*GSK-3 β*), ataxia telangiectasia mutated (*ATM*), and Mdm2 p53 binding protein homolog (*MDM2*) genes may play an important role in the effects of *PPP2R5C* knockdown in Jurkat T cells.

Introduction

T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) accounts for 10–15% of newly diagnosed ALL cases in children and 20–25% of ALL cases in adults. Although treatment outcome for patients with T-ALL has improved in recent years, relapsed T-ALL remains a challenge (Aifantis *et al.*, 2008). Monoclonal antibodies, gene inhibitors, and regulating microRNAs are promising tools for targeted cancer therapy (Peipp *et al.*, 2002; Sato and Yamochi, 2005; Ishida *et al.*, 2006; Ravandi *et al.*, 2006; Rodig *et al.*, 2006; Labbaye and Testa, 2012; Wang *et al.*, 2012; Zinzani *et al.*, 2012). However, most therapies have targeted B-cell malignancy and myeloid leukemia, and few targeted therapies are available for T-cell malignancies. For example, transforming *Mer* signals may contribute to T-cell leukemogenesis, and the regulation of *Mer* expression could be a novel therapeutic target for pediatric ALL therapy (Graham *et al.*, 2006). The

recent identification of *Notch1*-activating mutations in the majority of patients with T-ALL has stimulated interest in targeting the Notch signaling pathway for this disease (Palomero *et al.*, 2009). Further advances in the treatment of T-cell malignancies require the development of novel agents that target specific malignancies without significant toxicity.

In 2001, synthetic small interfering RNAs (siRNAs) were demonstrated to be capable of inducing RNAi in mammalian cells by Thomas Tuschl (Elbashir *et al.*, 2001). This discovery led to a surge in interest in harnessing RNAi for biomedical research and drug development. siRNA is a promising gene-targeting agent that has shown great potential, particularly in the field of cancer treatment (Devi, 2006; Oh *et al.*, 2009; Whitehead *et al.*, 2009). A combination of *c-raf* and *bcl-2* siRNA induced apoptosis in HL-60, U937, and THP cell lines and increased chemosensitivity to etoposide and daunorubicin (Cioca *et al.*, 2003). Based on the finding that B-cell chronic lymphocytic leukemia/lymphoma 11B (*BCL11B*)

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overexpression is detected in most T-cell malignancies (Oshiro *et al.*, 2006; Liu *et al.*, 2010; Huang *et al.*, 2011), a previous study demonstrated that the inhibition of *BCL11B* expression by *BCL11B*-siRNA leads to apoptosis in Molt-4, Jurkat, and huT78 cell lines (Grabarczyk *et al.* 2007).

Recently, we characterized the novel chromosomal translocation t(14;14)(q11;q32), which causes rearrangement of the T-cell receptor (TCR) α gene J7 segment (*TRAJ7*) with the *PPP2R5C* gene (unpublished data) in a case of Sézary syndrome at the molecular level. *PPP2R5C* is a regulatory B subunit of protein phosphatase 2A (PP2A), which is a major cellular serine/threonine phosphatase that affects the phosphorylation status of many proteins (Muneer *et al.*, 2002). The *PPP2R5C* gene encodes five differentially spliced variants: B56 γ 1, B56 γ 2, B56 γ 3, B56 γ 5, and B56 γ 6, whereas B56 γ 4 is identified only in mice. The locus for the functional *PPP2R5C* gene is at 14q32.2, whereas the nonfunctional B56 γ 1 pseudogene *PPP2R5C* is at 3p21.3 (Muneer *et al.*, 2002; Lee *et al.*, 2010). *PPP2R5C* plays a crucial role in cell proliferation, differentiation, and transformation based on its induction of the dephosphorylation of p53 at various residues (Shouse *et al.*, 2008). It has been reported that the dynamic nuclear distribution of the B56 γ 3 regulatory subunit controls the nuclear PP2A activity and may be responsible for the tumor-suppressive function of PP2A (Lee *et al.*, 2010). Recently, alterations in the expression pattern of *PPP2R5C* that are associated with malignant transformation have been characterized in lung cancer, and the *PPP2R5C* mutation F395C disrupts the B56 γ -p53 interaction (Shouse *et al.*, 2010).

We were also the first to find that *PPP2R5C* overexpression occurs in leukemias, including T-ALL (Zheng *et al.*, 2011). To confirm the role of *PPP2R5C* in proliferation and its molecular mechanism, we screened highly efficient and specific *PPP2R5C*-siRNAs, which target different exons in the human T-ALL cell lines Molt-4 and Jurkat, and a highly efficient *PPP2R5C*-siRNA was used further to evaluate its molecular mechanism in the proliferation inhibition of leukemic T cells.

Methods

Cell culture

The human T-cell leukemia cell lines Molt-4 and Jurkat (Institutes for Biological Sciences Cell Resource Center, Chinese Academy of Sciences, Shanghai, China) were grown in the Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco-BRL) with 10% fetal calf serum (FCS) (Sijiqing Co.) and maintained in a humidified incubator at 37°C and 5%

CO₂. All experiments were performed using cells in the exponential growth phase.

siRNA design and synthesis

The siRNAs *PPP2R5C*-siRNA1, *PPP2R5C*-siRNA2 (Chinese patent number: ZL 2011 1 0340411.1), and *PPP2R5C*-siRNA3 (Chinese patent number: ZL 201110337837.1), which target domains in the fourth, sixth, and between the eighth and ninth exons in the *PPP2R5C* gene (ACCESSION NM_178587), respectively, and a scrambled nonsilencing siRNA control (SC) were designed with online software (www.invitrogen.com) and synthesized by Invitrogen (Table 1). An Alexa Red Oligo (Invitrogen) was used to measure transfection efficiency.

Nucleofection

Cells were collected by centrifugation and resuspended at 2.5×10^6 cells for Molt-4 and Jurkat cells per 100 μ L of the appropriate Nucleofector™ kit solution (Amaxa Biosystems) (Huang *et al.*, 2011; Chen *et al.*, 2012; Zha *et al.*, 2012). Malignant T cells were nucleofected with 3 μ g of *PPP2R5C*-siRNAs or a control nonsilencing scrambled (SC) siRNA using the C-005 (Molt-4 cells) or X-001 (Jurkat cells) program of the Nucleofection Device II (Amaxa Biosystems). Mock-transfected cells nucleofected without siRNA were used as a negative control. After nucleofection, the cells were immediately mixed with 500 μ L of prewarmed culture medium and transferred into culture plates. The treated cells were incubated at 37°C for 3 days for cell proliferation, apoptosis, and microarray analyses. Three independent experiments for Molt-4 or Jurkat T cells were performed every 24 h.

RNA isolation, reverse transcription, real-time qRT-PCR, and microarray analysis

Total RNA was isolated from different samples (Molt-4 and Jurkat T cells) using TRIzol (Invitrogen). cDNA for qRT-PCR was synthesized using the Superscript II RNaseH reverse transcriptase kit (Invitrogen).

The expression level of *PPP2R5C* and the β 2-MG reference gene was determined by SYBR Green I real-time PCR. PCR was performed as previously described (Zheng *et al.*, 2011). The sequences for the primers used in qRT-PCR were as follows: *PPP2R5C*: 5'-GTAATAAAGCGGGCAGC AGG-3' (forward) and 5'-CAAAGTCAAAGAGGACGCAACA-3' (reverse) for *PPP2R5C* gene amplification and β 2M: 5'-CAGCAAGGAC TGGTCTTT CTAT-3' (forward) and β 2M 5'-GCGGCATCTTC AAAC CTC-3' (reverse) for β 2M gene amplification.

TABLE 1. SEQUENCES OF *PPP2R5C*-siRNA AND SCRAMBLED NONSILENCING siRNA CONTROL

No. of siRNA	Target (nt)	Sense (5'-3')	Antisense (5'-3')	Chinese patent number
<i>PPP2R5C</i> -siRNA1	574–598	UAGAGUCUCCAGAUUU CCAACCUAA	UUAGGUUGGAAAUCU GGAGACUCUA	
<i>PPP2R5C</i> -siRNA2	799–823	GCAUAGCAGAGUUACU GGAAAUAAU	AAUAUUUCCAGUAACU CUGCUAUGC	201110340411.1
<i>PPP2R5C</i> -siRNA3	991–1015	CAGUGGUGAUGGCACUU CUAAAUAA	UAUUUGAGAAGUGCC AUCACCACUG	201110337837.1
SC		UAGUCUACCUAGCUU AACCCAGUAA	UUACUGGGUUAAGCU AGGUAGACUA	

SC, scrambled nonsilencing siRNA control.

Total RNA (>3 µg) was sent for global gene expression profile analysis using the Affymetrix HG-U133 Plus 2.0 gene chip (Shanghai Biochip Co. Ltd). Affymetrix microarray analysis was performed using the Gene Spring GX11.0 software (Agilent Technologies) (Lai *et al.*, 2011; Huang *et al.*, 2011a; Chen *et al.*, 2012). Probe sets displaying a signal log ratio indicating an increase or marginal increase [i.e., log ratio $\geq 1(n)$] and the detection of an experimental group displaying a signal change with *P* represented up-regulated genes. Conversely, probe sets displaying a signal log ratio indicating a decrease or marginal decrease [i.e., log ratio $\leq -1(n)$] and the detection of a control group displaying a signal change with *P* represented downregulated genes. The resulting data were analyzed using the SBC Analysis System. After normalization and correction, the log₂ fluorescence intensity value for each gene was obtained (Huang *et al.*, 2011b). The fold change for all genes was calculated by comparing *PPP2R5C*-siRNA2- and SC-treated Jurkat T cells. A minimum twofold difference was considered significant.

Cell proliferation assays

The proliferation of Molt-4 and Jurkat T cells was indirectly assayed using the CCK-8 kit (Dojindo), which stains living cells. After transfection, approximately 5×10^4 Molt-4 or Jurkat (100 µL) and control cells were incubated in triplicate in 96-well plates. At 24, 48, and 72 h, the CCK-8 reagent (10 µL) was added to each well and incubated at 37°C for 2 h. The optical density at 450 nm was measured using an automatic microplate reader (Synergy4; Bio-Tek).

Apoptosis analysis

Molt-4 and Jurkat T cells transfected with *PPP2R5C*-siRNAs or Alexa Red Oligo were monitored from 48 to 72 h post-transfection using a Delta Vision high-resolution imaging system (Applied Precision, Inc.). The Molt-4 and Jurkat T cells (5×10^5) in each group were collected 48 h after transfection and then prepared with FITC-labeled anti-Annexin-V (BD Pharmingen) and propidium iodide (Kaiji) according to the manufacturers' protocol and measured by flow cytometry (Beckman Coulter). The results were analyzed using Windows MDI 2.9 software.

Statistical analyses

Statistical analyses were performed with paired *t*-tests and one-way analysis of variance using SPSS 13.0 statistical software. Kruskal-Wallis analysis was used to analyze the *PPP2R5C* gene mRNA levels in different samples. Differences were considered statistically significant at $p < 0.05$.

Results

PPP2R5C-specific siRNAs suppress *PPP2R5C* expression in Molt-4 and Jurkat T cells

We first verified the transfection efficiency with Alexa Red Oligo-transfected Molt-4 and Jurkat T cells, which was found to be $58.12\% \pm 14.14\%$ and $65.2\% \pm 10.3\%$, respectively (Fig. 1). To examine the knockdown of *PPP2R5C* expression in Molt-4 and Jurkat cells after siRNA treatment, *PPP2R5C* mRNA expression was analyzed by qRT-PCR 24, 48, and

FIG. 1. Alexa Red Oligo-transfected Molt-4 and Jurkat T cells and MOCK transfected control cells 10 h after transfection as measured by FCM (positive cells are shown in the P2 domain).

72 h after nucleofection. The expression level of *PPP2R5C* was significantly decreased in Molt-4 and Jurkat T cells treated with all three *PPP2R5C*-siRNAs compared with the control ($p < 0.05$) (Fig. 2) and the expression levels of *PPP2R5C* were obviously decreased (Figure S1; supplementary materials are available online at <http://www.liebertpub.com/dna>).

PPP2R5C suppression inhibits the proliferation of Molt-4 and Jurkat T cells

All three *PPP2R5C*-siRNAs were used to assess biological consequences. The proliferation rate of Molt-4 and Jurkat T cells transfected with different *PPP2R5C*-siRNAs was significantly decreased at 72 h compared with the control. In comparison with scrambled nonsilencing RNA-treated cells, Molt-4 and Jurkat T cells transfected with *PPP2R5C*-siRNAs had a significantly inhibited proliferation rate ($p < 0.05$) (Fig. 3). The inhibition rate was $49.23\% \pm 2.12\%$ for Molt-4 T cells treated with siRNA2 at 72 h and 48.87 ± 0.69 and $47.12\% \pm 0.72\%$ for Jurkat cells treated with siRNA1 and siRNA2, respectively, at 72 h. However, the transfected cells did not demonstrate a significant increase in Annexin V/PI-positive cells (apoptosis) at 48 or 72 h, and the apoptosis rate in cells from different

FIG. 2. Inhibition of *PPP2R5C* expression in Molt-4 and Jurkat cells by RNA interference. The expression of *PPP2R5C* in Molt-4 (A) and Jurkat T cells (B) treated with different *PPP2R5C*-siRNAs and control. A significant decrease in the *PPP2R5C* expression level was observed in all siRNA treatment groups. $*p < 0.05$. Color images available online at www.liebertpub.com/dna

FIG. 3. The inhibition of the proliferation of Molt-4 and Jurkat T cells transfected with *PPP2R5C*-siRNAs at 72 h as measured by the CCK-8 method. $*p < 0.05$ compared with scrambled, nonsilencing siRNA-treated cells. Color images available online at www.liebertpub.com/dna

groups was $12.80\% \pm 2.73\%$ (siRNA1), $2.11\% \pm 2.75\%$ (siRNA2), $13.35\% \pm 3.66\%$ (siRNA3), $8.76\% \pm 2.19\%$ (SC), $11.73\% \pm 2.11\%$ (MOCK), and $6.60\% \pm 1.88\%$ (NC) for Molt-4 T cells at 48 h, and $12.79\% \pm 1.39\%$ (siRNA1), $22.41\% \pm 1.76\%$ (siRNA2), $18.40\% \pm 6.36\%$ (siRNA3), $11.48\% \pm 2.77\%$ (SC), $16.60\% \pm 4.37\%$ (MOCK), and $7.38\% \pm 2.45\%$ (NC) for Jurkat T cells at 48 h. We have also checked the change of cell cycle of the siRNA-treated T cells by flow cytometry; there were no significant differences between different groups at 48 and 72 h after siRNA treatment and controls either in Jurkat or Molt-4 T cells (Figures S2 and S3).

Global gene expression profile in PPP2R5C-siRNA2-treated Jurkat T cells

To elucidate the gene expression profile of Jurkat T cells after *PPP2R5C* suppression, global gene expression analysis was performed by comparing the transcriptome profiles of *PPP2R5C*-siRNA2- and SC-treated Jurkat cells using Affymetrix HG-U133 Plus 2.0 arrays. There was a clear dysregulation in global gene expression in Jurkat T cells with *PPP2R5C* knockdown as demonstrated by the degree of differential expression between the *PPP2R5C*-siRNA2 and SC groups (Fig. 4A). Subsequently, the dysregulated genes were clustered based on their differential expression (two-fold up or down) and visualized using a heatmap (Fig. 4B). Gene Spring GX software analysis demonstrated that 439 genes (greater than twofold: 304 genes, greater than fourfold: 74 genes, greater than eightfold: 61 genes) were upregulated, and 524 genes (greater than twofold: 369 genes, greater than fourfold: 86 genes, greater than eightfold: 69 genes) were downregulated at least twofold in *PPP2R5C*-siRNA2-transfected Jurkat T cells compared with the SC control. Changes in signaling pathway genes closely related to cell proliferation, TCR signaling, Wnt signaling, calcium signaling, MAPK signaling, and p53 signaling were further analyzed by pathway analysis using the SBC Analysis System (Table 2).

Discussion

PPP2R5C plays a crucial role in cell proliferation, differentiation, and transformation based on its induction of the

FIG. 4. Microarray analysis after *PPP2R5C* knockdown in Jurkat T cells. **(A)** The Affymetrix data were clustered, and the red and green colors represent the expression levels that are increased or decreased with respect to the average expression across samples. **(B)** Scatter plots comparing the gene expression profiles of siRNA2- and siRNA control (SC)-transfected cells. The yellow dots represent genes absent in both samples, blue dots represent genes present in both samples, red dots represent genes upregulated, and green dots represent genes downregulated. Color images available online at www.liebertpub.com/dna

dephosphorylation of p53 at various residues (Shouse *et al.*, 2008) and may be responsible for the tumor-suppressive function of PP2A (Lee *et al.*, 2010). To confirm the role of *PPP2R5C* siRNA on the inhibition of leukemic T cells and its potential as a therapeutic agent, we compared different *PPP2R5C*-siRNAs that target different exon sequences and screened leukemic T cells with a highly efficient and specific *PPP2R5C*-siRNA.

Exogenous siRNA delivery always results in a transient RNAi effect. In general, RNAi effects are detected between 24 to 72 h after siRNA transfection. We demonstrated that the siRNAs effectively silenced *PPP2R5C* post-transcriptionally, and the control siRNA had no obvious influence 72 h after nucleofection. These results were not only confirmed by real-time PCR, but also by microarray analysis, which showed that *PPP2R5C* was downregulated 2.49- and 2.77-fold by two probe sets. siRNAs targeting different exon domains had different efficacies for *PPP2R5C* gene silencing and subsequent biological consequences. *PPP2R5C*-siRNA2, which targets exon six, demonstrated robust knockdown, whereas *PPP2R5C*-siRNA1 and *PPP2R5C*-siRNA3 had different effects on the different leukemic cell lines.

There are no reports regarding the effects of the suppression of *PPP2R5C* on changing cell biological functions. In this study, we demonstrated that the suppression of *PPP2R5C* by RNAi effectively inhibited the proliferation of the Molt-4 and Jurkat cell lines. However, unlike other reported siRNAs, such as *BCL11B*-siRNA and *Notch1*-siRNA (Guo *et al.*, 2009; Huang *et al.*, 2011), the suppression of *PPP2R5C* by RNAi could not significantly induce apoptosis, which was confirmed by gene expression profile analysis demonstrating that only a limited number of apoptotic genes are altered. Therefore, it is interesting to analyze the molecular mechanisms of the *PPP2R5C* siRNA-mediated suppression of cell proliferation.

To characterize the effects of *PPP2R5C* knockdown in Jurkat T cells, we analyzed gene expression profiles by gene chip. Data from global gene expression analysis demonstrated that approximately 1000 genes were differentially expressed; thus, we attempted to find genes involved in signaling pathways related to the downregulation of *PPP2R5C*.

Genes altered in the TCR signaling pathway

TCR engagement initiates intracellular signaling cascades that lead to T-cell proliferation, cytokine production, and differentiation into effector cells (Wilkinson *et al.*, 2005). Therefore, the TCR signaling pathway is the most important pathway in T-cell activation and proliferation (Laky *et al.*, 2006; Zha *et al.*, 2013), and according to the KEGG (Kyoto Encyclopedia of Genes and Genomes) website, there are 108 genes involved in the TCR signaling pathway (Pathway: map04660) (Kanehisa, 2002; Zha *et al.*, 2013). Our results demonstrated that only three genes, p21 protein (Cdc42/Rac)-activated kinase 1 (*PAK1*), mitogen-activated protein kinase kinase kinase 8 (*MAP3K8*), and glycogen synthase kinase 3 beta (*GSK-3 β*), were downregulated in *PPP2R5C*-siRNA-treated Jurkat T cells (Table 1). All three genes are also involved in the MAPK signaling pathway, and *GSK-3 β* is involved in the cell cycle pathway and is related to tumor proliferation (Varisli *et al.*, 2011). The downregulation of *GSK-3 β* is thought to decrease the phosphorylation of NFAT, while *MAP3K8* downregulation effects the inhibition of activator protein-1 (*AP-1*), which is critically modulated by post-translational modifications, particularly phosphorylation, which mediates not only TCR, but also MAPK cascades (Torgersen *et al.*, 2008). Therefore, our results indicate that the effect of the proliferation inhibition of Jurkat cells by *PPP2R5C* downregulation might be due to a common cell

TABLE 2. THE DETAILED LIST OF GENES WITH EXPRESSION CHANGES RELATED TO SIGNALING PATHWAYS IN JURKAT T CELLS AFTER PPP2R5C KNOCKDOWN

<i>Gene symbol</i>	<i>NCBI accession</i>	<i>Fold change</i>	<i>Description</i>	<i>Pathway</i>
MAP3K2	NM_006609	2.67	Mitogen-activated protein kinase kinase kinase 2	MAPK signaling pathway
MAP3K6	NM_004672	14.22	Mitogen-activated protein kinase kinase kinase 6	MAPK signaling pathway
MAP3K8	NM_005204	-2.08	Mitogen-activated protein kinase kinase kinase 8	MAPK signaling pathway /T-cell receptor signaling pathway
DDIT3	NM_001195053	-2.32	DNA-damage-inducible transcript 3	MAPK signaling pathway
FGF7	NM_002009	3.68	Fibroblast growth factor 7	MAPK signaling pathway
CACNB2	NM_000724	6.34	Calcium channel, voltage-dependent, beta 2 subunit	MAPK signaling pathway
RasGRP2	NM_001098670	2.30	RAS guanyl releasing protein 2	MAPK signaling pathway
NF1	NM_000267	-2.09	Neurofibromin 1	MAPK signaling pathway
STMN1	NM_001145454	-9.63	Stathmin 1	MAPK signaling pathway
NF-κB2	NM_001077493	2.24	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2	MAPK signaling pathway
PAK1	NM_001128620	-2.88	p21 protein (Cdc42/Rac)-activated kinase 1	MAPK signaling pathway/T-cell receptor signaling pathway
ATM	NM_000051	-2.01	Ataxia telangiectasia mutated	Cell cycle/p53 signaling pathway /Apoptosis
MDM2	NM_002392	16.51	Mdm2 p53 binding protein homolog	Cell cycle/p53 signaling pathway
GSK-3β	NM_001146156	-2.19	Glycogen synthase kinase 3 beta	Cell cycle/T-cell receptor signaling pathway/Wnt signaling pathway
PTEN	NM_000314	2.76	Phosphatase and tensin homolog	p53 signaling pathway
ADRA1B	NM_000679	-2.33	Adrenergic, alpha-1B-, receptor	Calcium signaling pathway
ATP2A3	NM_005173	9.84	ATPase, Ca++ transporting, ubiquitous	Calcium signaling pathway
DRD5	NM_000798	-2.13	Dopamine receptor D5	Calcium signaling pathway
RYR3	NM_001036	38.04	Ryanodine receptor 3	Calcium signaling pathway
HRH2	NM_001131055	8.93	Histamine receptor H2	Calcium signaling pathway
HTR7	NM_000872	-4.56	5-hydroxytryptamine (serotonin) receptor 7	Calcium signaling pathway
ITPR1	NM_002222	2.00	Inositol 1,4,5-triphosphate receptor, type 1	Calcium signaling pathway
P2RX1	NM_002558	-2.31	Purinergic receptor P2X, ligand-gated ion channel, 1	Calcium signaling pathway
GRIN2C	NM_000835	-2.12	Glutamate receptor, ionotropic, N-methyl D-aspartate 2C	Calcium signaling pathway
CALM1	NM_001166106	4.52	Calmodulin 1	Calcium signaling pathway
ADCY1	NM_021116	2.35	Adenylate cyclase 1 (brain)	Calcium signaling pathway
ADCY2	NM_020546	-5.51	Adenylate cyclase 2 (brain)	Calcium signaling pathway
ADCY9	NM_001116	2.41	Adenylate cyclase 9	Calcium signaling pathway
Dkk1	NM_012242	-2.16	Dickkopf homolog 1	Calcium signaling pathway
APC	NM_000038	2.37	Adenomatous polyposis coli	Wnt signaling pathway
APC2	NM_005883	3.13	Adenomatous polyposis coli 2	Wnt signaling pathway
PPP2R5C	NM_178587	-2.49	Protein phosphatase 2, regulatory subunit B', gamma	Wnt signaling pathway
TCF7L1	NM_031283	-7.21	Transcription factor 7-like 1 (T-cell specific, HMG-box)	Wnt signaling pathway
Dam1	NM_014992	-3.90	Dishevelled associated activator of morphogenesis 1	Wnt signaling pathway
CFLAR	NM_001127183	2.39	CASP8 and FADD-like apoptosis regulator	Apoptosis
C5F2Rβ	NM_000395	-31.26	Colony stimulating factor 2 receptor, beta, low affinity	Apoptosis
PRKAR1A	NM_002734	2.50	Protein kinase, cAMP-dependent, regulatory, type I, alpha	Apoptosis
XIAP	NM_001167	2.06	X-linked inhibitor of apoptosis	Apoptosis
CDC14A	NM_003672	-2.05	Cell division cycle 14 homolog A	Cell cycle
STAG1	NM_005862	2.32	Stromal antigen 1	Cell cycle
CDKN1A	NM_000389	2.42	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Cell cycle/p53 signaling pathway
CDKN1C	NM_000076	2.31	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	Cell cycle
ORC5	NM_002553	-3.75	Origin recognition complex, subunit 5	Cell cycle

proliferation pathway, such as the MAPK pathway, rather than the TCR signaling pathway.

Genes altered in the p53 signaling pathway

p53 is an important tumor suppressor protein, in humans, whose main functions are involved in preventing genome mutations and regulating the cell cycle. In response to DNA damage and other stress signals, p53 is highly modified post-translationally, which increases its stability and promotes its activation and nuclear localization (Shouse *et al.*, 2010; Yang and Phiel, 2010; Isin *et al.*, 2012). B56 γ -containing PP2A is an important p53 regulatory enzyme that directly regulates DNA damage-induced p53 stabilization. In response to DNA damage, B56 γ -containing PP2A triggers Thr55 dephosphorylation on p53 and promotes its transcriptional activation (Li *et al.*, 2007; Shouse *et al.*, 2008). In addition, B56 γ -containing PP2A could dephosphorylate Bcl2 and prevent the proteasome-dependent degradation of Bcl2, which inhibits p53 and resists apoptosis (Lin *et al.*, 2006). Thus, B56 γ -containing PP2As regulate the p53 network at multiple levels. In this study, the expression of p53 was not directly downregulated in Jurkat T cells by inhibiting *PPP2R5C* expression; however, the *MDM2* gene, which is an important negative regulator of the p53 tumor suppressor, was upregulated 16.51-fold (Uhrinova *et al.*, 2005), while the ataxia telangiectasia mutated (*ATM*) gene, a positive regulator of the p53 that triggers p53 Ser15 phosphorylation and promotes its transcriptional activation, was downregulated 2.1-fold (Ditch *et al.*, 2012). Recently, it is proved that ATM directly phosphorylates and specifically regulates B56 γ 3, B56 γ 2, and B56 δ , after DNA damage, the phosphorylation of B56 γ 3 at Ser510 leads to an increase in B56 γ 3-PP2A complexes, and direction of the PP2A phosphatase activity toward the substrate p53, activating its tumor-suppressive functions (Shouse *et al.*, 2011). Both the MDM2 and ATM proteins act as an E3 ubiquitin ligase, which binds to the N-terminal domain of the p53 protein and inhibits p53 transcriptional activation (Vassilev *et al.*, 2004). This result suggests that *PPP2R5C* knockdown in Jurkat T cells may lead to a decrease in the transcriptional activation of the p53 protein and p53 function by down-regulating *ATM* and upregulating *MDM2*.

Genes altered in the canonical Wnt/ β -catenin signaling pathway

The canonical Wnt/ β -catenin signaling pathway plays an important role in the differentiation, polarization, and survival of mature T cells (Gattinoni *et al.*, 2010). β -catenin is a pivotal signaling molecule in the canonical Wnt/ β -catenin signaling pathway, and its function as a coactivator that cooperates with diverse DNA binding partners, such as the T-cell factor (TCF)/lymphoid enhancer binding factor (LEF) proteins, remodels chromatin and orchestrates transcriptional programs (Arce *et al.*, 2006). In the presence of the Wnt signal, the disheveled Wnt/receptor complex is recruited to promote the phosphorylation of the β -catenin destruction complex, which consists of the scaffold proteins adenomatous polyposis coli (APC), axin, GSK-3 β , and casein kinase 1. Phosphorylation leads the accumulation of β -catenin, and the accumulated β -catenin in its de- or underphosphorylated form is then translocated into the nucleus, where it interacts

with TCF/LEF factors to regulate gene expression (MacDonald *et al.*, 2009; Xue *et al.*, 2012). It has been well established that B56s are upstream regulators of β -catenin in the canonical Wnt/ β -catenin signaling pathway. B56s could interact with APC, promote the degradation of β -catenin, and inhibit the transcription of β -catenin target genes in mammalian cells (Seeling *et al.*, 1999). Our data indicate that in addition to *PPP2R5C*, two genes upstream of β -catenin, GSK-3 β and APC, were down- or upregulated, respectively, and one downstream gene *TCF7L1* was downregulated. Our data suggest that the knockdown of *PPP2R5C* by siRNA did not directly effect the expression of β -catenin, but it might indirectly effect the reduction in the accumulation of β -catenin by the up- and downregulation of β -catenin regulatory factors and inhibit the expression of the TCF/LEF gene. Down-regulation of the TCF/LEF gene could result in a decrease in T-cell development or activation. However, *TCF1* was also described as a T-cell-specific tumor suppressor gene in addition to its established role as a Wnt-responsive transcription factor (Tiemessen *et al.*, 2012), which appears to be in contrast with the present results. Whether there are different functions for different TCF family members requires further investigation.

Genes altered in the other signaling pathways

In this study, we also analyzed the differential regulation of genes involved in the MAPK, calcium, apoptosis and cell cycle signaling pathways. Many genes were upregulated or downregulated; however, it is hard to conclusively determine which are the direct results of *PPP2R5C* knockdown in Jurkat T cells. This finding is in agreement with results demonstrating insignificant differences in apoptosis and cell cycle genes in Jurkat T cells after *PPP2R5C* knockdown.

In conclusion, our findings provide evidence for the effect of proliferation inhibition in malignant T cells by *PPP2R5C* knockdown and characterize its relative molecular mechanism. *PPP2R5C* might be involved in the p53 and canonical Wnt/ β -catenin signaling pathways. The differential expression of the GSK-3 β , *ATM*, and *MDM2* genes may play an important role in the effects of *PPP2R5C* downregulation. Moreover, GSK-3 β plays a direct role in the stimulatory modification of the p65/RelA transcription factor, which targets NF- κ B p50/52 in NF- κ B signaling. Overall, *PPP2R5C* downregulation results in the inhibition of Jurkat T-cell proliferation, which involves numerous pathways, and decreased GSK-3 β may be a critical factor in this process. However, further validation of the differentially regulated genes and their relative protein expression levels is needed.

Authors' Contributions

Y.L. contributed to the concept development and study design. Y.C., S.L., Q.S., X.Z., H.Z., L.Y., S.C., X.W., and B.L. performed the laboratory studies. Y.L. and Y.C. coordinated the study and helped draft the manuscript. All authors read and approved the final manuscript.

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Disclosure Statement

The authors declare that they have no competing interests.

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