Liu et al. Journal of Hematology & Oncology (2015) 8:34
DOI 10.1186/s13045-015-0125-5

JOURNAL OF HEMATOLOGY & ONCOLOGY

LETTER TO THE EDITOR Open Access

Alteration of gene expression profile following *PPP2R5C* knockdown may be associated with proliferation suppression and increased apoptosis of K562 cells

Sichu Liu[1†], Qi Shen[1,2†], Yu Chen[1,3], Chengwu Zeng[1], Changshu Cao[1], Lijian Yang[1], Shaohua Chen[1], Xiuli Wu[1], Bo Li[1] and Yangqiu Li[1,3*]

Abstract

We reported that knockdown of *PPP2R5C* by siRNA led to proliferation inhibition and apoptosis induction in K562 cells. In this study, we further characterized the gene expression profiles after *PPP2R5C* suppression by microarray analysis. Genes which participate in the MAPK, PI3K/AKT, and JAK/STAT pathways, were mainly altered in the K562 cells. We propose that the mechanism for proliferation inhibition and increased apoptosis of K562 cells following *PPP2R5C* suppression may be related to the alteration of expression profiles of *BRAF, AKT2, AKT3, NFKB2* and *STAT3* genes.

Keywords: *PPP2R5C*, CML, BCR-ABL, Gene expression profile

Findings

Overexpression of *PPP2R5C* is associated with the malignant transformation of several kinds of leukemia [1]. Recently we characterized the effects of downregulating *PPP2R5C* on the proliferation and apoptosis of K562 and Jurkat cells using different siRNAs which were targeting *PPP2R5C*. Significant proliferation inhibition was confirmed both in K562 and Jurkat cells, whereas apoptosis induction could only be observed in K562 and K562R cells [2,3].

To further investigate the gene expression profile, *PPP2R5C*-siRNA991-treated K562 cells were collected at 48 h post transfection when *PPP2R5C* mRNA was most suppressed [2]. Gene expression profiles were determined and analyzed by Affymetrix microarrays as reported (See Additional file 1 for methods and materials) [3,4]. Overall, 2,586 genes were upregulated and 2,601 genes were downregulated at least two-fold, when *PPP2R5C*-siRNA991 and SC-treated expression data were compared. We also found both the *Bcr* and *Abl* genes were downregulated (fold change: −1.23 and −1.53, respectively), suggesting that *PPP2R5C* is closely related to the BCR-ABL-mediated pathway. Besides that, there were changes in genes involved in different signaling pathways closely related to cell proliferation and apoptosis (Table 1, Figure 1A and B).

Aberrant BCR-ABL tyrosine kinase activity plays a crucial role in the pathogenesis of CML [5,6]. Moreover, abnormal interactions between the BCR-ABL oncoprotein and other molecules lead to the disruption of the major cellular processes, including the MAPK, JAK/STAT and PI3K/AKT signaling pathway, which can result in the dysregulation of proliferation and apoptosis [7].

In the MAPK signaling pathway, 67 genes were differentially expressed including 20 upregulated and 47 downregulated genes. The significantly downregulated genes including *BRAF, MAP2K2, ELK1, NFKB2, FOS,* and *JUN*. Downregulated *BRAF* might decrease the expression

* Correspondence: yangqiuli@hotmail.com
†Equal contributors
[1]Institute of Hematology, Jinan University, Guangzhou 510632, China
[3]Key Laboratory for Regenerative Medicine of Ministry of Education, Jinan University, Guangzhou 510632, China
Full list of author information is available at the end of the article

Table 1 Cell proliferation and apoptosis genes altered after *PPP2R5C*knockdown in K562 cells in microarray analysis

Gene symbol	NCBI accession	Fold change	Description	Pathway
BRAF	NM_004333	−2.24	v-raf murine sarcoma viral oncogene homolog B1	MAPK signaling pathway
MAP2K2	NM_030662	−2.39	mitogen-activated protein kinase kinase 2	MAPK signaling pathway
ELK1	NM_001114123	−2.65	ELK1, member of ETS oncogene family	MAPK signaling pathway
FOS	NM_005252	−3.12	FBJ murine osteosarcoma viral oncogene homolog	MAPK signaling pathway
JUN	NM_002228	−4.88	jun proto-oncogene	MAPK signaling pathway
NFKB2	NM_001077493 MAPK signaling pathway/AKT signaling pathway	−2.81	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	
AKT2	NM_001626	−2.72	V-akt murine thymoma viral oncogene homolog 2	MAPK signaling pathway/AKT signaling pathway
AKT3	NM_005465	−12.47	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	MAPK signaling pathway/AKT signaling pathway
CRKL	NM_005207	−2.14	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	MAPK signaling pathway/AKT signaling pathway
IL6ST	NM_001190981	−2.13	interleukin 6 signal transducer (gp130, oncostatin M receptor)	Jak-STAT signaling pathway
STAT3	NM_003150 Jak-STAT signaling pathway	−5.08	signal transducer and activator of transcription 3 (acute-phase response factor)	
MDM2	NM_002392	2.26	Mdm2 p53 binding protein homolog	AKT Signaling Pathway/p53Signaling Pathway
ATM	NM_000051	−2.30	ataxia telangiectasia mutated	p53Signaling Pathway

and phosphorylation of the downstream proteins MAP2K2, ELK1, NFKB2, FOS and JUN (Figure 1C) [8]. As a consequence, the major effects of the proliferation inhibition in *PPP2R5C*-siRNA991-treated K562 cells might be via the BRAF inhibition.

There were alterations involved in the PI3K/AKT signaling pathway including 6 upregulated and 6 downregulated genes (Figure 1D). *PPP2R5C* suppression predominantly resulted in *MDM2* upregulation and downregulation of *CRKL*, *AKT2*, *AKT3*, and *NFKB2*. PI3K activates AKT kinases and causes the phosphorylation of downstream factors that regulate the AKT-mediated cellular apoptotic machinery [8,9], while downregulation of *CRKL* weakens BCR-ABL binding to PI3K, leading to reduced AKT phosphorylation. Moreover, a reduction in NFKB2 might be directly linked to the induction of apoptosis [10], and MDM2, a negative regulator of p53, might indirectly affect apoptosis [11]. Therefore, it is thought that AKT2, AKT3 and NFKB2 might be involved in apoptosis induction in K562 cells after *PPP2R5C* inhibition.

In the JAK/STAT signaling pathway, 28 genes were differentially expressed, including 16 upregulated and 12 downregulated genes (Figure 1E). The downregulated genes *IL6ST* and *STAT3* may play important roles in cell proliferation through inhibition of the IL-6/JAK/STAT3 pathway, and STAT3, which is a signal transducer, plays a key role in cell survival in human hematopoietic malignancies [12]. Thus, *PPP2R5C* suppression might have effect on the JAK/STAT pathway through *STAT3* downregulation, leading to proliferation inhibition in K562 cells.

Because the mediation of cell proliferation, differentiation, and transformation functions of *PPP2R5C* is based on its induction of p53 dephosphorylation at various residues [13,14], a dominant alteration in p53 pathway was found for *ATM*, which had 2.3-fold downregulation, and *MDM2*, which was upregulated 2.26-fold. These results are similar to our previous finding in Jurkat cells in which we showed that proliferation was suppressed by *PPP2R5C*-siRNA. It is thought that ATM downregulation and MDM2 upregulation might lead to a decreased transcriptional activation level for p53, suggesting that the *PPP2R5C*-mediated p53 function might use the same signaling pathway in different leukemia cells.

In conclusion, we characterized altered expression profile of genes related to the BCR-ABL signaling pathway in *PPP2R5C*-siRNA-treated K562 cells. The mechanism of *PPP2R5C*-suppression-mediated inhibition of proliferation and increased apoptosis in K562 cells may be related to the MAPK, PI3K/AKT, JAK/STAT pathways through *BRAF, AKT2, AKT3, NFKB2* and *STAT3* downregulation. However, further validation of the altered genes and related proteins is needed.

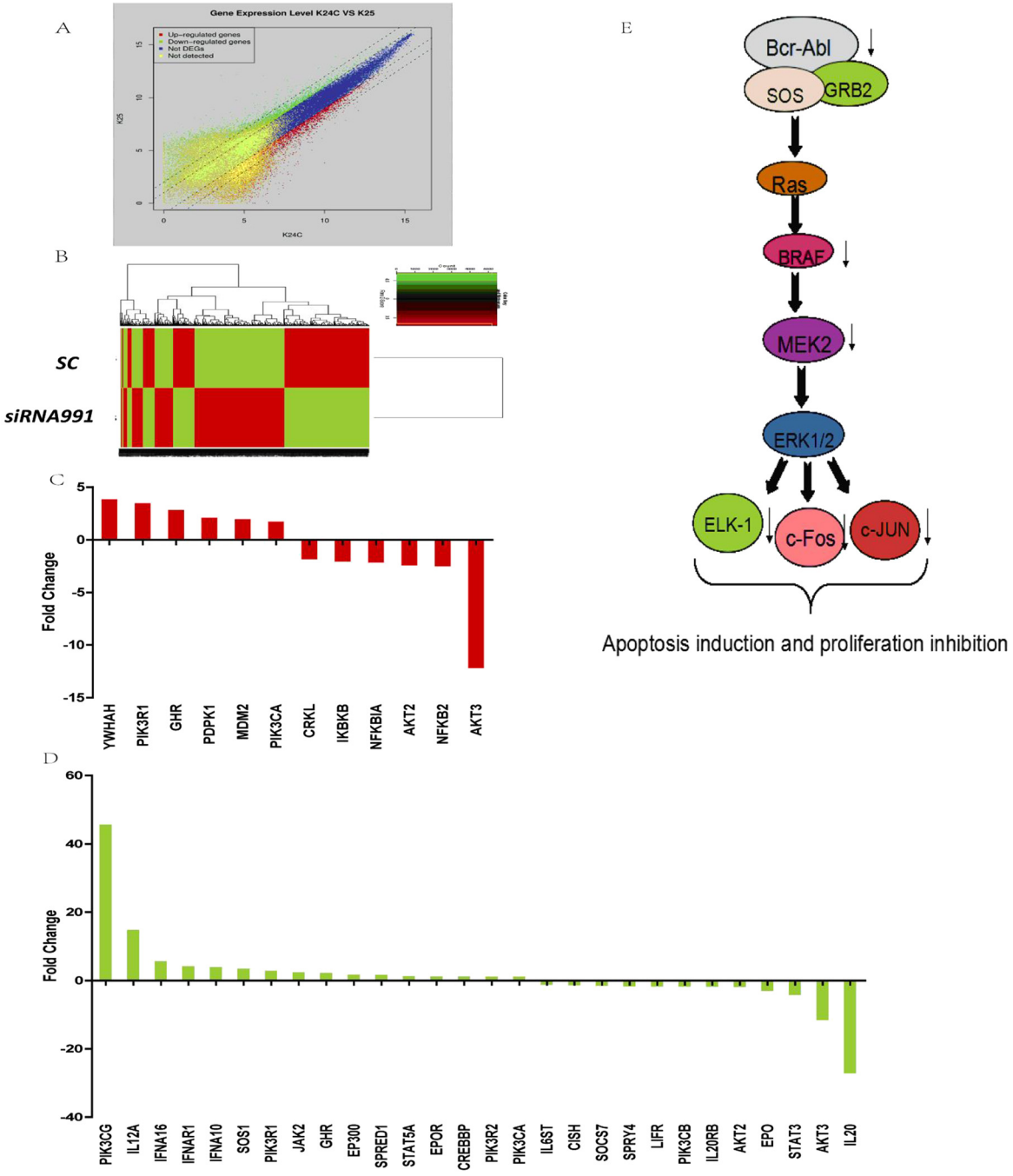

Figure 1 Microarray analysis for gene expression profiles of K562 cells after transfection with PPP2R5C-siRNA991. (**A**) Scatter plots comparing the gene expression profiles of siRNA991 and scrambled control (SC) transfected cells. The yellow dots represent genes undetected in both samples, blue dots represent genes present in both samples, red dots represent upregulated genes, and green dots represent downregulated genes. (**B**) The Affymetrix data were clustered, and the red and green colors represent the expression levels increased or decreased, respectively, with respect to the average expression across all samples. (**C**) PI3K/AKT signaling pathway genes differentially expressed in K562 cells after *PPP2R5C* suppression. (**D**) JAK/STAT signaling pathways genes differentially expressed in K562 cells after *PPP2R5C* suppression. (**E**) Schematic model of the BCR-ABL-mediated BRAF-MEK-FOS-JUN signaling pathway due to PPP2R5C suppression in K562 cells (modified from reference [8]).

Additional file

Additional file 1: Methods and materials.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
YQL contributed to concept development and study design. SCL and QS performed the cell culture, nucleofection, and RNA isolation and data analysis. YC, CWZ, CSC, XLW and BL helped to array data analysis, LJY and SHC helped to cell culture and collect samples. YQL and SCL coordinated the study and helped draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This study was supported by grants from National Natural Science Foundation of China (U1301226), a collaborate grant for HK-Macao-TW of the Ministry of Science and Technology (2012DFH30060), the Guangdong Science & Technology Project (2012B050600023) and Science and Technology Innovation Key Project of Guangdong Higher Education Institutes (kjcxzd1013).

Author details
[1]Institute of Hematology, Jinan University, Guangzhou 510632, China. [2]Department of Hematology, The Second Clinical Medical college (Shenzhen People's Hospital), Jinan University, Shenzhen 518020, China. [3]Key Laboratory for Regenerative Medicine of Ministry of Education, Jinan University, Guangzhou 510632, China.

Received: 20 February 2015 Accepted: 4 March 2015
Published online: 12 April 2015

References
1. Zheng H, Chen Y, Chen S, Niu Y, Yang L, Li B, et al. Expression and distribution of PPP2R5C gene in leukemia. J Hematol Oncol. 2011;4:21.
2. Shen Q, Liu S, Chen Y, Yang L, Chen S, Wu X, et al. Proliferation inhibition and apoptosis induction of imatinib-resistant chronic myeloid leukemia cells via PPP2R5C down-regulation. J Hematol Oncol. 2013;6:64.
3. Chen Y, Liu S, Shen Q, Zha X, Zheng H, Yang L, et al. Differential gene expression profiles of PPP2R5C-siRNA-treated malignant T cells. DNA Cell Biol. 2013;32:573–81.
4. Zha X, Chen S, Yang L, Shi L, Li B, Wu X, et al. Upregulated TCRzeta enhances interleukin-2 production in T-cells from patients with CML. DNA Cell Biol. 2012;31:1628–35.
5. Sweet K, Zhang L, Pinilla-Ibarz J. Biomarkers for determining the prognosis in chronic myelogenous leukemia. J Hematol Oncol. 2013;6:54.
6. Okabe S, Tauchi T, Katagiri S, Tanaka Y, Ohyashiki K. Combination of the ABL kinase inhibitor imatinib with the Janus kinase 2 inhibitor TG101348 for targeting residual BCR-ABL-positive cells. J Hematol Oncol. 2014;7:37.
7. Cilloni D, Saglio G. Molecular pathways: BCR-ABL. Clin Cancer Res. 2012;18:930–7.
8. De Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N. The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: role in cancer pathogenesis and implications for therapeutic approaches. Expert Opin Ther Targets. 2012;16 Suppl 2:S17–27.
9. Panigrahi S, Stetefeld J, Jangamreddy JR, Mandal S, Mandal SK, Los M. Modeling of molecular interaction between apoptin, BCR-Abl and CrkL–an alternative approach to conventional rational drug design. PLoS One. 2012;7:e28395.
10. Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature. 1999;401:82–5.
11. Mayo LD, Donner DB. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc Natl Acad Sci U S A. 2001;98:11598–603.
12. Sansone P, Bromberg J. Targeting the interleukin-6/Jak/stat pathway in human malignancies. J Clin Oncol. 2012;30:1005–14.
13. Shouse GP, Cai X, Liu X. Serine 15 phosphorylation of p53 directs its interaction with B56gamma and the tumor suppressor activity of B56gamma-specific protein phosphatase 2A. Mol Cell Biol. 2008;28:448–56.
14. Saha MN, Qiu L, Chang H. Targeting p53 by small molecules in hematological malignancies. J Hematol Oncol. 2013;6:23.