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

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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).

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...
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.

### Table 1 Cell proliferation and apoptosis genes altered after PPP2R5Cknockdown 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_00114123    | −2.65       | ELK1, member of ETS oncogene family MAPK signaling pathway |
| FOS         | NM_005252      | −3.12       | FB1 murine osteosarcoma viral oncogene homolog MAPK signaling pathway |
| JUN         | NM_002228      | −4.88       | jun proto-oncogene MAPK signaling pathway |
| NFKB2       | NM_001077493   | −2.81       | nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100) MAPK signaling pathway/akt signaling pathway |
| 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      | −5.08       | signal transducer and activator of transcription 3 (acute-phase response factor) Jak-STAT signaling pathway |
| 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 |

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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]).
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, LIY 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.

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