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LETTER TO THE EDITOR

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Upregulated TCRζ improves cytokine secretion in T cells from patients with AML

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Abstract

Previous studies indicated that upregulating *TCRζ* partially recovers T cell function in patients with leukemia. In this study, we characterized the cytokine profile of *TCRζ*-transfected T cells from acute myeloid leukemia (AML) patients by Quantibody®Array Glass Chip. Firstly, the significantly lower expression of TCRζ in $CD3^+/TCRζ^+$ cells from AML patients was found. Increased secretion of IL-2, IL-8, IL-10, IL-13, IFN-γ, TNF-α, GM-CSF, growth-regulated oncogene (GRO), MIP-1b, and regulated on activation, normal T cell expressed and secreted (RANTES) could be detected in T cells from AML patients after TCRζ upregulating. We concluded that upregulating TCRζ in T cells from AML can alter the secretion profile of cytokines and chemokine which are involved in T cell proliferation and activation.

Keywords: Acute myeloid leukemia, T cells, TCRζ, Cytokine, Chemokine

Findings

Acute myeloid leukemia (AML) is an aggressive disease with an unfavorable prognosis [1–3]. T cell immunodeficiency is a common characteristic in hematological malignancies which may be due to defective TCRζ. Previous studies showed that *TCRζ*upregulation could be induced in $CD3^+$T cells from AML patients by IL-2, IL-7, and IL-12 [4]. In this study, we characterized the secretion profile of cytokines and chemokines related to T cell activation in TCRζ-IRES2-EGFP-transfected T cells from AML patients after TCRζupregulation.

First, significantly lower TCRζ expression in $CD3^+/TCRζ^+$ cells in AML (2.89 ± 2.6 %, $n = 10$) was found in comparison with healthy individuals (87.38 ± 15.67 %, $n = 10$) ($p < 0.001$) (Fig. 1a–c). This result further supported our previous finding that T cell immunodeficiency might be due to low TCRζ signaling in T cells [5–8].

$CD3^+$T cells were sorted from PBMCs from four AML patients (Additional file 1: Table S1) who had TCRζ deficiency and then transfected with TCRζ-IRES2-EGFP or IRES2-EGFP, respectively, by nucleofection [9]. Significant upregulation of TCRζ in TCRζ-IRES2-EGFP-transfected $CD3^+$T cells was confirmed. Similar results were found in *TCRζ* downstream target factor Zap-70 (Fig. 1d–f). Thus, *TCRζ* gene transfection could directly upregulate TCRζ and Zap-70 in T cells from AML patients as previously found in CML [9].

Forced TCRζ chain expression can reverse TCR/CD3-mediated signaling abnormalities and defective IL-2 production in T cells [9, 10]. In this study, we used Quantibody®Array Glass Chip (www.raybiotech.com) to quantitatively measure 20 human cytokines and chemokines in supernatants from TCRζ-IRES2-EGFP-transfected and IRES2-EGFP-transfected T cells from AML patients (Additional file 2). Increased secretion of IL-2, IL-8, IL-10, IL-13, IFN-γ, TNF-α, GM-CSF, growth-regulated oncogene (GRO), MIP-1b, and regulated on activation, normal T cell expressed and secreted (RANTES) and decreased secretion of IL-5 were found, while the secretion level of IL-1α, IL-1β, IL-4, IL-6, and IL-12 had no obvious change after TCRζupregulation. Moreover, the changes in the secretion levels of IL-10, MCP-1, MIP-1a, MMP-1, and VEGF were different in different AML samples (Fig. 2). After TCRζ transfection, the IFN-γ secretion level was increased in all samples in the TCRζ-IRES2-EGFP group (median 71.46 pg/mL) compared with the pIRES2-EGFP

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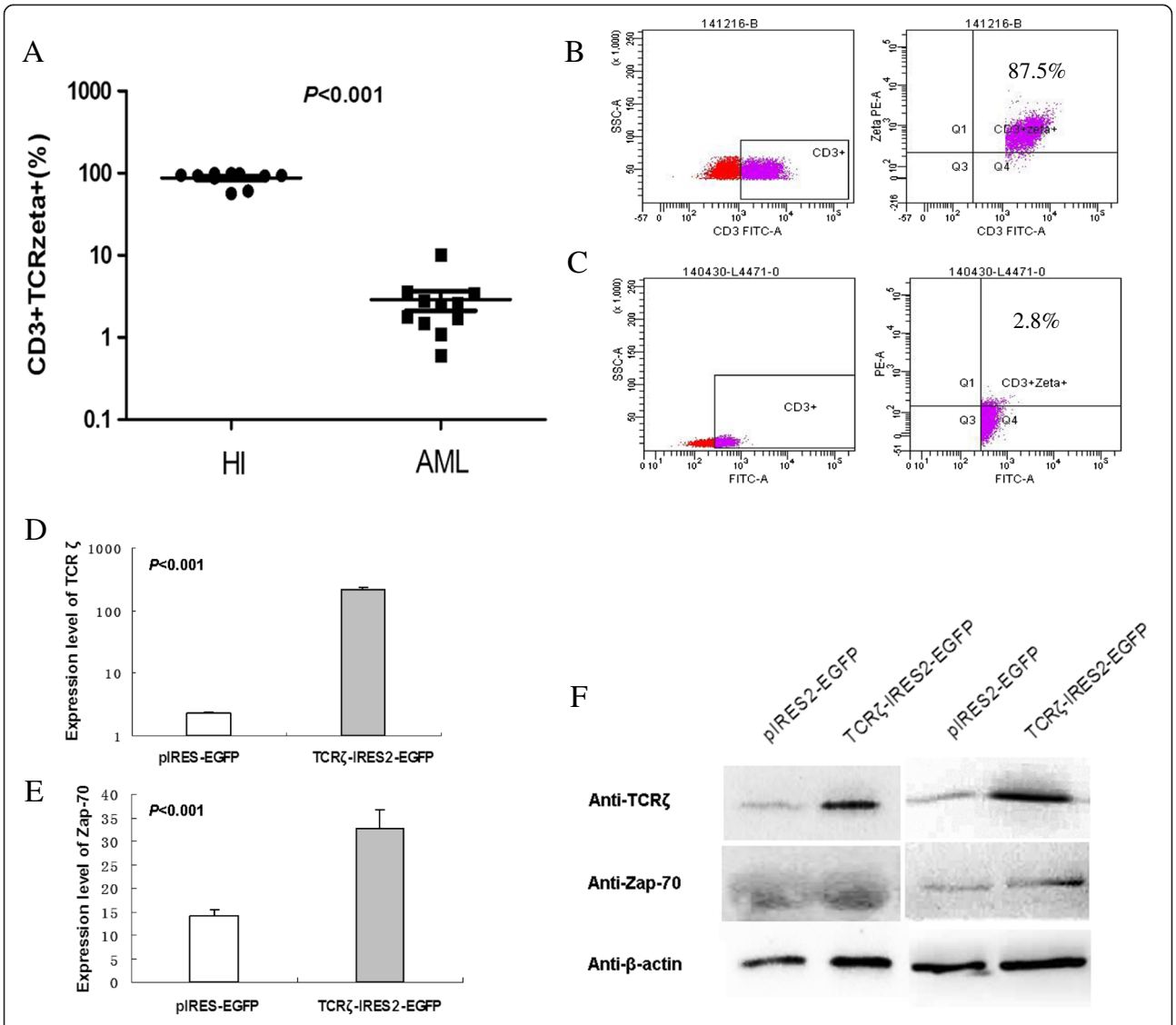

Fig. 1 Expression of $CD3^+/TCR\zeta^+$ cells in PBMCs and expression of TCRζ and Zap-70 in TCRζ-transfected $CD3^+$ T cells from patients with AML. **a** MFI of $CD3^+/TCR\zeta^+$ in PBMCs from AML patients and healthy individuals (*HI*) (n =10). **b** Percentage of $CD3^+/TCR\zeta^+$ cells in PBMCs from a healthy individual. **c** Percentage of $CD3^+/TCR\zeta^+$ in PBMCs from a patient with AML. **d** TCRζ gene expression levels. **e** Zap-70 gene expression level in TCRζ-transfected $CD3^+$ T cells and control cells. **f** TCRζ and Zap-70 protein expression in transfected $CD3^+$ T cells from two AML samples and control cells

group (median 42 pg/mL) ($P = 0.253$) because the basal level of IFN-γ in T cells from different AML patients was relatively different, ranging from 18.89 to 169.41 pg/mL in control cells and from 54.02 to 335.33 pg/mL in TCRζ-IRES2-EGFP cells. Thus, it could be understood that the increased secretion of IFN-γ was not statistically different in this study even though there was an obvious change in its level. Similar characteristic was found in TNF-α secretion level (Additional file 3: Figure S1). Interestingly, we found that the level of GM-CSF was significantly increased (21.63 ± 15.19 pg/mL for TCRζ-IRES2-EGFP cells vs. 1.96 ± 1.83 pg/mL for pIRES2-EGFP cells) ($p = 0.045$) (Fig. 2), and IL-13, which is secreted by activated T cells and has synergistic effects with GM-CSF and G-CSF, was also upregulated after TCRζ gene transfection (Fig. 2). Recently, increasing data have shown that GM-CSF has a variety of effects on the immune system including the activation of T cells, maturation of dendritic cells, and the ability to promote humoral and cell-mediated responses; thus, it has been incorporated into immunotherapy strategies [11, 12].

In conclusion, we characterized the profile of cytokines and chemokines secretion in T cells after TCRζ gene

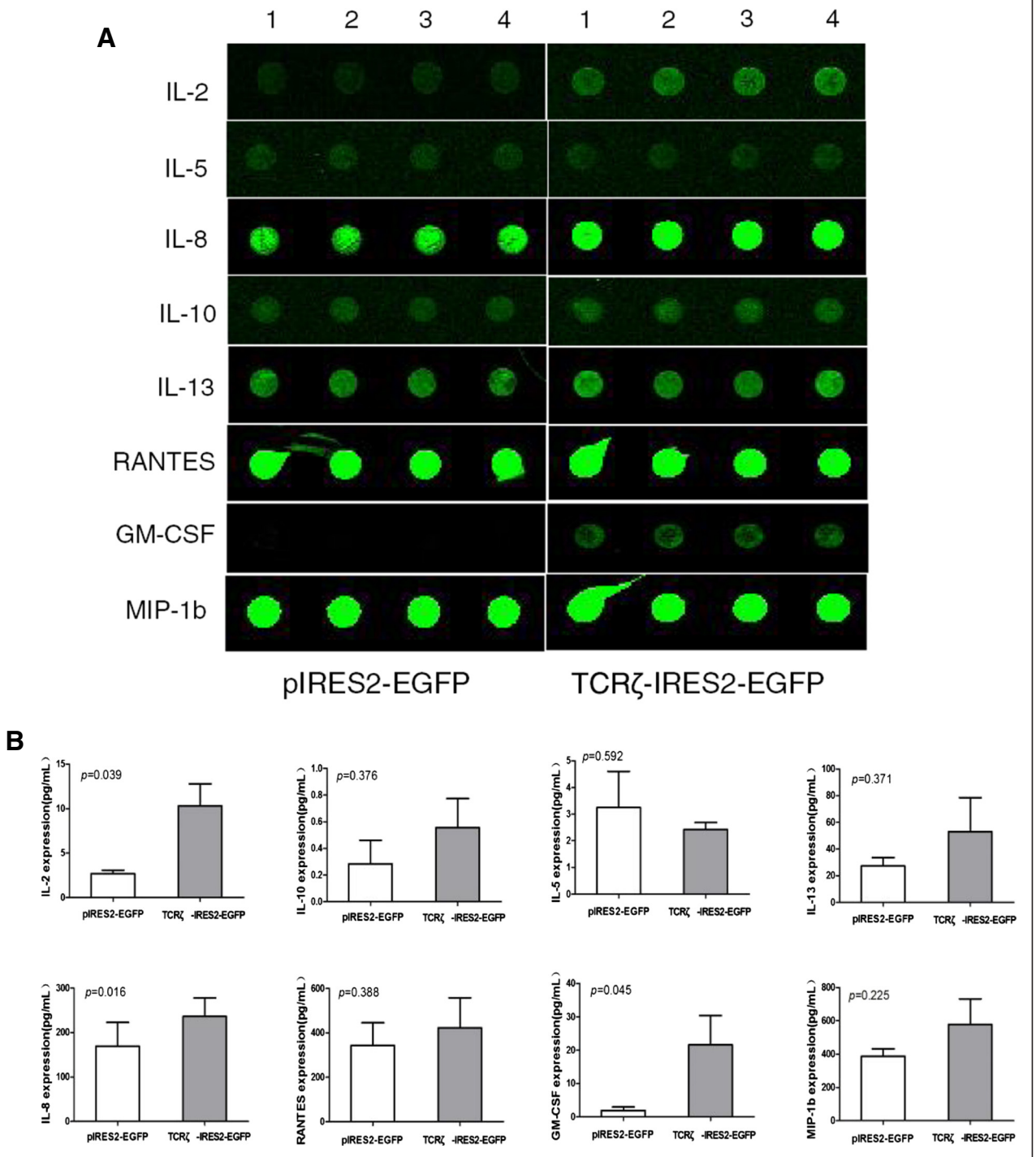

Fig. 2 Detection of the IL-2, IL-5, IL-8, IL-10, IL-13, RANTES, GM-CSF, MIP-1b, IFN-γ, and TNF-α level secreted from T cells from AML patients using Quantibody® array. **a** Fluorescence intensity (concentration) from laser scanner results. *1–4*: four parallel wells for each sample. **b** The level of IL-2, IL-5, IL-8, IL-10, IL-13, RANTES, GM-CSF, and MIP-1b secreted from T cells from four cases with AML

transfection. Most cytokines related to T cell proliferation and activation, such as IL-2, IFN-γ, and TNF-α, had increased secretion after TCRζ upregulating. Moreover, some of the Th1-associated CC subfamily chemokines, such as CCL4 and CCL5, may contribute to T cell activation via TCRζ upregulation. These results may further support the idea of the effects of upregulating TCRζ in T cell immunity.

Additional files

Additional file 1: Table S1. Clinical data of AML patients.

Additional file 2: Materials and methods.

Additional file 3: Figure S1. Detection of the IFN-γ and TNF-α level in T cells from AML patients using Quantibody® array. A: Laser scanner fluorescence intensity (concentration) results. 1–4: samples from four cases with AML. B: The secretion level of IFN-γ and TNF-α in T cells from four cases with AML.

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

Authors' contributions
YQL and SHC contributed to the concept development and study design. SHC, XFZ, LS, LLZ, LJY, BL, and XLW performed the experiments and analyzed the data. JZ, ZT, YHL, and KEZ were responsible for clinical diagnoses and performed clinical data acquisition. YQL, SHC, and XFZ coordinated the study and helped draft the manuscript. All authors read and approved the final manuscript.

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