Growth and Differentiation Effects of Homer3 on a Leukemia Cell Line

Zheng Li1&, Hui-Ying Qiu1&, Yang Jiao2, Jian-Nong Cen1, Chun-Mei Fu1, Shao-Yan Hu3, Ming-Qing Zhu1, De-Pei Wu1, Xiao-Fei Qi1,4*

Abstract

The Homer protein family, also known as the family of cytoplasmic scaffolding proteins, which include three subtypes (Homer1, Homer2, Homer3). Homer3 can regulate transcription and play a very important role in the differentiation and development for some tissues (e.g. muscle and nervous systems). The current studies showed that Homer3 abnormal expression changes in acute myeloid leukemia (AML). Forced expression of Homer3 in transfected K562 cells inhibited proliferation, influenced the cell cycle profile, affected apoptosis induced by As2O3 through inhibition of Bcl2 expression, and also promoted cell differentiation induced by 12-O-tetradecanoylphorbol-acetate (TPA). These results showed that Homer3 is a novel gene which plays a certain role in the occurrence and development of AML.

Keywords: Homer3 - leukemia cell line - cell proliferation - apoptosis - differentiation

Introduction

The Homer protein family, also known as the family of cytoplasmic scaffolding proteins, which include three subtypes (Homer1, Homer2, Homer3) (Brakeman et al., 1997; Xiao et al., 1998). Homer3 can regulate transcription and play a very important role in the differentiation and development for some tissues (e.g. muscle and nervous systems) (Ishiguro et al., 2004; Shiraishi et al., 2004; Bortoloso et al., 2006). Current studies showed that Homer3 were abnormal expressed in blasts in AML (Stirewalt et al., 2008) and myelodysplastic syndrome (MDS) (data not shown). And decreased expression for Homer3 was associated with a poor outcome in AML (Valk et al., 2004). Our study will try to reveal the function of Homer3 in the leukemia cell line.

Materials and Methods

RNA isolation and RT-qPCR (real time quantitative PCR)

Total mRNAs were extracted from leukemia cell lines (K562, NB4, HL-60, SHI-1) using Trizol (Invitrogen, USA) as described by the manufacturer’s instructions. mRNA was quantified by BioPhotometer plus (eppendorf, Germany) at the wavelength of 260 nm. Ensured the concentration of RNA was 0.5 µg/µl, The 2 µg total RNA were reverse-transcribed with an MMLV reverse transcriptase, and the resulted cDNA was subjected for evaluating Homer3 expression, qRT-PCR was performed with an ABI 7500 Real Time PCR System (Applied Biosystems, USA). The PCR reaction conditions were as follows: initially 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. All samples were normalized to the internal control (ABL) and the Homer3 expression level was calculated using the \(2^{-\Delta\Delta CT} \) method.

Cell culture and transfection

The K562 leukemia cell line was cultured in IMDM containing 20% FCS. Plasmid of pcDNA-Homer3 was transfected into K562 cells and stable transfectants were selected for their G418 resistance (800 mg/ml). Empty vector was also transfected as a control.

Western blot analysis

Over-expressed Homer3 clones were detected by Western blot analysis. The stable cells were lysed using the IP (immunoprecipitation) lysate in the presence of PMSF proteinase inhibitor (Beyotime, China). After denatured in 5XLaemmli sample buffer at 100°C for 5 min, total proteins were electrophoresed on 15% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and transferred onto polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% non-fat milk in PBST 2 h before incubation with the primary antibody. The primary antibody used mouse polyclonal to Homer3 (Abcam, USA). GAPDH as a loading control. After incubated with secondary antibody
for 1 h at room temperature, the signals were visualized with enhanced chemiluminescence (ECL) (Beyotime, China).

**Cell proliferation in liquid culture assays**

The cells were counted using Cell Counting Kit-8 (CCK-8) beginning in 96-well plates at $2 \times 10^3$ cells/well; 20 μl CCK-8 solution was added to each well, the final volume of 200 μl and the IMDM containing 20% FCS, after 2 h of incubation with 37°C and 5% CO₂, the optical absorbance at 450 nm was measured. Each experiment was performed three times.

**Flow cytometric analysis for cell cycles**

After culture without FCS for 3-6 h, 20% FCS were added into the medium followed by continuous culture for 24 h, then harvest the cells and washed twice with ice-cold PBS before incubation at 4°C 30 min in dark conditions with a monoclonal mouse anti-human CD15-PE antibody, the PE-conjugated anti-mouse-IgG as a control tube.

**Statistical analysis**

Each experiment was performed three times; data were presented as mean ± standard deviation (SD), where applicable, and differences were evaluated using Student’s t-tests. $P$ values < 0.05 were considered statistically significant.

**Results**

*Homer3 can inhibit cellular proliferation of K562 cell*

qRT-PCR was used to detect the expression of Homer3 in several cell lines. We found that Homer3 mRNA expression was significantly lower in K562 cell line than in other cell lines (NB4, HL-60, SHI-1) (Figure 1A). For low expressed of Homer3 gene, K562 cell line was selected for our study finally. K562 cell line, also known as acute erythroleukemia (M6) cell line, was isolated from patients with chronic myelocytic leukemia (CML) at blast phase. To examine the effect of Homer3 on cell proliferation, the transfected K562 cell line with Homer3 (K562/Homer3) and control clone (Vector) were selected with annexin-V and necrotic cells (stained with both annexin-V and PI).
Homer3 enhanced apoptosis of K562 cells induced by As$_2$O$_3$.

In order to define the difference of As$_2$O$_3$-induced apoptosis between K562/Homer3 and Vector, the cells were incubated in the presence of As$_2$O$_3$ (0.8 μmol) at 37°C for 3 h. The cells were stained by annexin-V and analyzed by flow cytometry. Over thirty-three percent of the cells in the stable transfected K562 clones over-expressed Homer3 were positive for annexin-V staining while very few annexin-V positive stained cells were detected in control clone (Figure 2A). The result is significant (P<0.05) (Figure 2B).

To elucidate the relevance of the Homer3 gene to the apoptosis pathway, we analyzed the protein levels of both Bcl2 and Bax in cells exposed to As$_2$O$_3$. In stable K562 clones over-expressed Homer3 the protein Bcl2 was markedly reduced compared to the control clones (P<0.05) (Figure 2C), while no difference was found in the Bax protein level between these two groups (data not shown).

Homer3 influenced cell cycle

The cell cycle distribution was analysed by flow cytometry. The results showed that there were a markedly higher number of the K562/Homer3 clone cells in G2/M phase than Vector (P<0.05) (Figure 3B).

Homer3 promoted cell differentiation distribution upon TPA induction

CD15 is a surface molecule of mature granulocyte and monocyte. The cell differentiation degree analysis by flow cytometry and the cells treated by TPA, results showed a significantly higher proportion of the K562/Homer3 cells than Vector (Figure 4B).

Discussion

Acute myeloid leukemia (AML), a deadly form of hematopoietic malignancies, is a group of heterogeneous diseases with considerable diversity in terms of clinical behavior and prognosis, it is the most common malignant cancer in children and young people (Weltermann et al., 2004; Pasqualucci et al., 2006; Falini et al., 2007; Mills et al., 2009; Burnett et al., 2011). Gene-expression detection technology in the hematopoietic cell of AML patients have been applied in scientific research and clinical testing, in hope of identifying candidate genes which may refer to the development and progression of AML and marker of diagnosis or prognosis (Deluany et al., 2003; Weltermann et al., 2004; Gonzalez Garcia et al., 2006; Foran, 2010; Flach et al., 2011). For example, in the passed several years, the nucleophosmin (PNM1) mutations with normal cytogenetics has been identified a prognostic factors in AML patients (Schnittger et al., 2005), more and more genes would probably provide biomarkers in the future.

In the present, researching the function of Homer3 mainly in the nervous system and the immune cell. But in some previous data showed that Homer3 were abnormal expression changed in blasts in AML and MDS. Although the significance of Homer3 abnormal expression changes in leukemic blasts is unknown, further analyses found a significant association between Homer3 gene expression levels and prognosis (Stirewalt et al., 2008). The AML patients can be divided into three groups by the abnormal of cytogenetics has been identified a prognostic factors in AML patients (Schnittger et al., 2005), more and more genes would probably provide biomarkers in the future.
In our experiments, the forced over-expressed of the Homer3 gene in K562 cells can suppress cellular proliferation, promote cell apoptosis, differentiation and affect cell cycle significantly. So we think that the hematopoietic cell clones in leukemia of favorable groups which over-expressed Homer3 might weaken a growth advantage through these effects. All of these can explain it is over-expressed in MDS and leukemia of the low risk group, while in high-risk group of the patients displayed decreased expression. In conclusion, we found the up-regulation of Homer3 expression might be an important event of pathogenesis and prognosis in AML. We believe that it will become a new ways of gene therapy in AML patients in the future.

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References

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