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# Over-expression of *Hlx* homeobox gene in DC2.4 dendritic cell enhances its maturation and antigen presentation

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ABSTRACT

*Hlx* as a Th1-specific transcription factor, it appears to drive maturation of Th1 and IFN-γ secretion in cooperation with T-bet. In this study, we established a stable *Hlx*-over-expressed dendritic cell line (DC2.4/*Hlx*), and investigated the possible effect of *Hlx* gene on maturation of dendritic cell-line (DC2.4). Results shown that over-expressed *Hlx* in DC2.4 up-regulated the transcription and expression of IFN-γ, increased the expression of maturation makers including CD40, CD80, CD86, MHC-I and MHC-II. Functional assay for DC2.4/*Hlx* showed that over-expressed *Hlx* increased the expression level of interleukin-12 in the supernatant and decreased DC endocytosis when cells were incubated in vitro. Furthermore, using a syngeneic T cell activation model, we found that DC2.4/*Hlx* could obviously present ovalbumin (OVA) antigen to T cell in OVA pre-immunized mice.



## 1. Introduction

*Hlx* is a homeobox gene, was originally isolated from a murine pre-B-lymphocyte cell line [1,2]. Hlx is very important not only in hematopoietic cells, but also in normal intestinal and hepatic development in mice [3]. In immune system, Hlx is a Th1-special transcription factor that interacts specifically with T-bet. Hlx and T-bet synergistically promote IFN-γ expression when co-expressed. Thus, as a cofactor of T-bet, Hlx appears to enhance the activities of T-bet and then favor Th1 differentiation [4]. The co-engagement of T-bet and Hlx actively suppressed Th2 commitment. Hlx down-regulates the IL-4 receptor expression in naive CD4+ T cells [5]. Over-expressed Hlx resulted in the aberrant expression of IFN-γ in normal CD4+ T cells during the differentiation under Th2-polarizing conditions [6,7]. Therefore, Hlx played an important role in the regulation of IFN-γ production by T cell. Interestingly, in NK cells Hlx was a negative regulator of IFN-γ production and its inhibitory function was achieved at least in part through the proteasomal degradation of STAT4 [8]. Therefore, Hlx may play the different role in different systems or cells.

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) for the initiation of antigen (Ag)-specific immune responses as well as maintenance of tolerance to self-antigens [9]. In addition, DCs was also directly involved in regulating other innate immunity such as promoting activation of NK cells and their effector function [10,11]. However, the role of Hlx in DCs functions remains unknown. In the present study, we introduced exogenous *Hlx* gene into a dendritic cell line DC2.4, established a stable DC2.4/*Hlx* cell-line. Our results showed that over-expression of Hlx in DC2.4 cells up-regulated production of IFN-γ, enhanced their maturation and antigen-presenting function.

## 2. Materials and methods

### 2.1. Cell culture

The immature murine dendritic cell line DC2.4 (H-2$^b$), DC2.4/*EGFP* and DC2.4/*Hlx* were cultured in RPMI1640 medium, supplemented with 100 U/ml penicillin, 100 mg/L streptomycin, 2 mmol/L L-glutamine, and 10% FBS (GIBCO). The transfected cell lines were pulsed with 0.6 g/L G418 to eliminate negative cells.

### 2.2. Transfection and selection of stable clones

The DC2.4 cells were transfected by *Hlx* with liposomes (lipofectamine™ 2000, Invitrogen). $4 \times 10^5$ DC2.4 cells in 500 μl complete RPMI1640 medium without antibiotics were plated in 24-well plates. Next day when the cells were at 60–70% confluent, *Hlx* was transfected according to the instruction of the manufac-

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turer. The medium was changed into 100 μl serum-free medium before transfection. The 100 μl DNA/lipofectamine complexes that were prepared at the ratio of 1:3 (μl) were added to each well and then mixed gently by rocking the plate back and forth, followed by incubating at 37 °C in a $CO_2$ incubator for 6 h. The medium was changed by 200 μl new complete RPMI1640 medium. The cells were passaged at 1:10 with fresh growth medium 24 h after transfection, and then selected by adding selective medium (contain 600 mg/L G418) in the following day. The target clones were selected on the basis of their resistance to G418 and expression of EGFP. The Hlx high-expressed DC2.4 was assessed by Real-time PCR and Western blot.

### 2.3. Total RNA isolation and Real-time PCR

Total RNA was prepared using Trizol (Invitrogen), and reversely transcribed with first strand cDNA synthesis kit ReverTraAce-a-™ (TOYOBO Co., Ltd., Life Science Department) according to the manufacturer's instructions. RT-PCR was performed by the comparative threshold cycle ($^{\Delta}C_T$) method and normalized to β-actin. Sequences of the primers are as follows: 5′-CTC GTG GTC CCG TGC TGT CTT TTC-3′ and 5′-GTT CCC TCA GTC CGT TCC GTG TCG-3′ for mouse Hlx, 5′-ATG CCA GGG AAC CGC TTA T-3′ and 5′-CAG ATG CGT ACA TGG ACT CAA A-3′ for mouse T-bet, 5′-CTG TGG GCT GTA CTA CAA GCT TCA-3′ and 5′-ACC CAT GGC GGT GAC CAT GC-3′ for mouse GATA3, 5′-AAG CGT CAT TGA ATC ACA CC-3′ and 5′-CGA AAT CAG CAG CGA CTC CTT AT-3′ for mouse IFN-γ, 5′-CGA GGT CAC AGG AGA AGG-3′ and 5′-TGC TCT TTA GGC TTT CCA G-3′ for mouse IL-4, 5′-CTC ACC TGT GAC ACG CCT GA-3′ and 5′-CAG GAC ACT GAA TAC TTC TC-3′ for mouse Il-12p40, 5′-CCT CAG TTT GGC CAG GGT C-3′ and 5′-CAG GTT TCG GGA CTG GCT AAG-3′ for mouse Il-12p35, 5′-TGG AAT CCT GTG GCA TCC ATG AAA C-3′ and 5′-TAA AAC GCA GCT CAG TAA CAG TCC G-3′ for mouse β-actin. PCR were set up with the SYBR *Premix Ex Taq*™ II (TaKaRa Biotechnology Co., Ltd., Dalian, China). Data were collected using the Rotor-gene 6000 real-time system (Corbett Life Science) and analyzed using the relative quantification (based on the relative expression of target genes vs. β-actin reference genes). Transcripts of each gene relative to the control gene were determined with the $2^{\Delta}$Ct method.

### 2.4. Western blot

Whole cells were lysed in lysis buffer (150 mM NaCl, 20 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1 mM $Na_3VO_4$, 1 mM sodium fluoride, 0.5% Doc, 1% Triton X-100, and 1% Nonidet P40). The cell lysates were boiled with 2× loading buffer for 5 min and

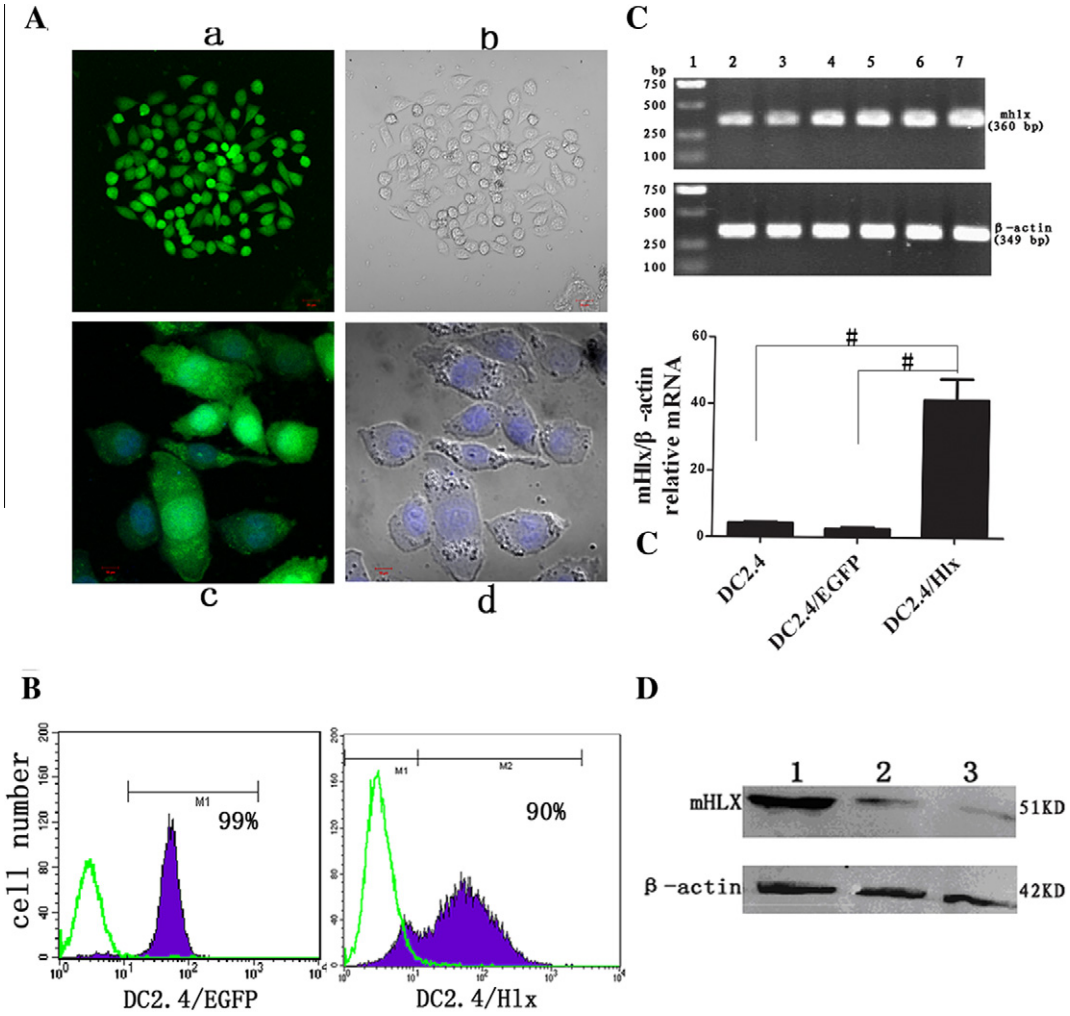


**Fig. 1.** Establishment of stable *Hlx*-overexpressed dendritic cell line (DC2.4-*Hlx*). (A) Expression and location of EGFP in stable DC2.4-*Hlx* clone, a and c showed the fluorescence intensity and localization of EGFP expression in DC2.4-Hlx detected by confocal microscopy; b and d appeared original state of DC2.4-*Hlx*. (B) The fluorescence ratio expressed in DC2.4-*Hlx* was identified by FACS. (C and D) Indicated the *Hlx* high-expressed in DC2.4-*Hlx* assessed by Real-time PCR and Western blot, upper half of figure C showed the *Hlx* expression in different cell clones, lane 1 DI2000 marker, lane 2 DC2.4, lane 3 DC2.4/EGFP, lane 4–7 different DC2.4/*Hlx* clone, and the lower half showed the statistical analysis of *Hlx* mRNA (mean ± SD, $n = 5$), #represent $p < 0.01$.