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Research paper

Functional analysis of the quantitative expression of a costimulatory molecule on dendritic cells using lentiviral vector-mediated RNA interference

Liang Zhang, Michele Procuik, Tielan Fang, Sam Kam-Pun Kung *

Department of Immunology, University of Manitoba, Room 417, Apotex Center, 750 McDermot Avenue, Winnipeg, Manitoba, Canada R3E 0T5

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ABSTRACT

The increasing number of co-stimulatory molecules identified on dendritic cells (DC) to date highlights the complex regulation of co-stimulatory signals in T cell activation. We previously established a single lentiviral vector system to stably express short hairpin RNA (shRNA) to induce RNA interference (RNAi) in cell lines and primary T cells. We reasoned that the choice of shRNA target sequences in the lentiviral vector system would also allow us to regulate different levels of surface expression of a co-stimulatory molecule stably and reproducibly. In this study, we first demonstrated that lentiviral vectors delivered RNA interference in DC without functional impairments. We used CD40 as a target co-stimulatory molecule to demonstrate the feasibility of using lentiviral vectors in delivering different shRNA target sequences to genetically modify DC that expressed different levels of CD40. We provided functional data to further demonstrate that quantitative expression of CD40 on LPS-stimulated DC have different functional outcomes on Ag-specific T cell responses in vitro. Collectively, we developed a simple system that will allow us to examine functional significance(s) of the quantitative and/or qualitative expression of a single or multiple co-stimulatory molecule(s) on DC.

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1. Introduction

Dendritic cells (DC) are professional antigen-presenting cells of the immune system. They have the ability to take up, process and present antigens (Ag) to resting lymphocytes in draining lymph nodes. The importance of DC in initiation of T cell immunity and tolerance is firmly established (Steinman et al., 2003). Functionally mature DC are highly efficient at priming adaptive immune responses against viruses, pathogens and endogenous tumors (Banchereau and Steinman, 1998); whereas steady state "immature" DC are capable of uptaking and presenting self-Ag to tolerate autoreactive T cells (Wilson et al., 2003; Bonifaz et al., 2002; Hawiger et al., 2001; Scheinecker et al., 2002; Steinman et al., 2000). Phenotypic analysis of the surface expression of co-stimulatory molecules has been routinely used in defining mature and "immature" DC. The increasing number of co-stimulatory molecules identified to date, however, highlights the complex regulation of co-stimulatory signals.

Development of a simple system that will modulate levels of surface expression of co-stimulatory molecules on DC will allow us to understand the functional significance(s) of the quantitative and/or qualitative expression of a single or multiple co-stimulatory molecule(s) on DC.

RNA interference (RNAi) is an innate cellular process that involves multiple RNA–protein interactions (Fire, 1999; Hannon, 2002). Its gene silencing activity is activated when a double-stranded RNA (dsRNA) molecule of greater than 19 duplex nucleotides enters the cells, causing degradation of both the dsRNA and single stranded RNA (endogenous mRNA) of identical sequences (Fire, 1999; Hannon, 2002). We and others have selected the most potent RNAi sequence for efficient gene silencing of target genes in specific cell types.

Abbreviations: DC, Dendritic cells; shRNA, Short hairpin RNA; LPS, Lipopolysaccharide; Ag, Antigen; RNAi, RNA interference; DsRNA, Double stranded RNA; Th, T helper.

* Corresponding author. Department of Immunology, University of Manitoba, 750 McDermot Avenue, Room 417 Apotex Center, Winnipeg, Manitoba, Canada R3E 0T5. Tel.: +1 204 480 1301; fax: +1 204 789 3921.
E-mail address: kung@cc.umanitoba.ca (Sam K.P. Kung).

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Transient transfection of short synthetic dsRNA in DC demonstrated the feasibility of applying this technique in the studies of DC functions (Li et al., 2004; Gu et al., 2006; Hill et al., 2003; Laderach et al., 2003; Liu et al., 2004; Orabona et al., 2005; Li et al., 2007). A single lentiviral vector system has been established to stably express short hairpin RNA (shRNA) to induce RNA interference (RNAi) in cell lines and primary T cells (An et al., 2003; Qin et al., 2003). In contrast to the transient transfection studies, the lentiviral vector system supports stable and long-term expression of shRNA in the modified cells that might prove useful in the development of stable, genetically defined DC to study the role of co-stimulatory molecules in functional definition of DC in vivo. As the sequence encoded in the dsRNA contributes to the potency of the dsRNA in degradation of target mRNA (Reynolds et al., 2004), we reasoned that the choice of shRNA target sequences in the lentiviral vector system would also allow us to regulate different levels of surface expression of a co-stimulatory molecule stably and reproducibly.

In the present study, we established the feasibility of using lentiviral vectors in delivering stable shRNA in the manipulation of DC functions in vitro and in vivo. We used a number of shRNA target sequences to demonstrate that lentiviral vectors delivered RNA interference in DC without functional impairments. We used CD40 as a target co-stimulatory molecule to demonstrate the feasibility of generating DC that express different levels of CD40. We provided functional data to further demonstrate that quantitative expression of CD40 on LPS-stimulated DC has different functional outcomes on Ag-specific T cell responses in vitro.

CD40 type I transcript NM_011611

ATGgtgtctttgcctcggctgtgcgcgctatggggctgcttgttgacagcggtccatctagggcagtgtgttacgtgcagtgacaaac
agtacctccacgatggccagtgctgtgatttgtgccagccaggaagccgactgacaagccactgcacagctcttgagaagacccaatg
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Fig. 1. RNAi sequences against mouse CD40 gene. GenBank accession no. NM011611, *Mus musculus* CD40 antigen, transcript variant 1 sequence was shown. Targeting sequences used in each lentiviral constructs were shown. pLKOCD40 clones (66243, nucleotide 2136–2156, GCAGGGTACTGGCTAAATAAA), (66244, nucleotide 1606–1626, CCAAAGGATAATGAGATGTTA) were obtained from Open Biosystems (Huntsville, AL); FG12-siCD40#1, nucleotide 2502-2522, TACCATTTAGTTCTTGAATAA. FG12-siCD40#2, nucleotide 1590–1610, AAAGGTGGTCAAGAAACCAAA. FG12-siCD40#3, nucleotide 2449–2469 GTGGTGTATTGTAGAAATTAT; FG12-siCD40#4, nucleotide 1431–1451, CTGGACAAGCTGTGAGGATAA. Start and stop codons were in bold.

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