Expression of T399I and D299G polymorphisms of TLR4 gene in colorectal cancer cell line by flowcytometery

Davoodi H (PhD)\textsuperscript{1}, Hashemi SR (PhD)\textsuperscript{2}, Seow HF (PhD)\textsuperscript{3}

\textsuperscript{1}PhD in Immunology, Faculty of Medicine, Golestan University of Medical Sciences, Golestan, Iran.
\textsuperscript{2}Assistant Professor, Faculty of Animal Sciences, Gorgan University of Agricultural Sciences and Natural Resources.
\textsuperscript{3}Professor, Department of Pathology, Medicine Faculty, UPM University, Malaysia.

Abstract

Background and Objective: Toll-like receptors (TLRs) have been discovered as the most important receptors in innate immunity. One of the most important TLRs is TLR4, the key receptor for the LPS component of gram-negative bacteria. Two polymorphisms, D299G (rs4986790) and T399I (rs4986791), in TLR4 gene are associated with a decreased response to LPS. This study was done to estimate the expression of different polymorphisms of TLR4 gene in colorectal cancer cell line by flowcytometry.

Materials and Methods: In this laboratory study, the HCT116 cells were transfected with plasmids containing different variants of TLR4 gene including; Flag-tagged-TLR4 wild type, flag-tagged D299G and T399I Using TurboFect transfection reagent. Transfection efficiency was evaluated by GFP plasmid. Expression of different variants of TLR4 was assessed in transfected cells by flowcytometry. Data were analyzed using SPSS-11.5 and chi-square test.

Results: TLR4 was detected on HT29 and CaCo2 cell lines at low levels. HCT116 cells did not express detectable amounts of TLR4 by flowcytometry prior to transfection. Gene transfer efficiency for GFP plasmid was about 80% in HCT116 cells by flowcytometry and microscopic analysis. TLR4 expression and LPS responsiveness significantly was higher in HCT116 cells which were transfected with wild type TLR4 gene compared to non-transfected and mutant transfected cells (P<0.05).

Conclusion: Lower expression of TLR4 on cells with mutant TLR4 showed that these polymorphisms affect on expression patterns of TLR4 on colon cancer cells.

Keywords: TLR4 gene, Polymorphism, LPS, HCT116 cell line

* Corresponding Author: Davoodi H (PhD), E-mail: homdavoodi@yahoo.com

Received 3 April 2012 Revised 15 September 2012 Accepted 11 November 2012