Original Paper

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

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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 flowcytometery.

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 flowcytometery. 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 flowcytometery prior to transfection. Gene transfer efficiency for GFP plasmid was about 80% in HCT116 cells by flowcytometery 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

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

This paper should be cited as: Davoodi H, Hashemi SR, Seow HF. [Expression of T399I and D299G polymorphisms of *TLR4* gene in colorectal cancer cell line by flowcytometery]. J Gorgan Uni Med Sci. 2013; 15(2): 47-53. [Article in Persian]