Expression Profile of Notch Receptors and Notch Ligands in Human Tonsillar Follicular Helper T Cell

การแสดงออกของนอทช์รีเซปเตอร์และนอทช์ลิแกนด์ในฟอลลิคูลาเฮลเปอร์ทีเซลล์จากต่อมทอนซิลของมนุษย์

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บทคัดย่อ

วิถีสัญญาณนอทช์เป็นวิถีสัญญาณที่สำคัญต่อการสื่อสารไปจนถึงการเจริญเติบโตและการทำงานของเซลล์ ฟอลิคูลาเฮลเปอร์ทีเซลล์จัดเป็นทีเซลล์กลุ่มผู้ช่วย พบได้ในต่อมน้ำเหลืองทุติยภูมิ มีหน้าที่ในการช่วยและกระตุ้นให้บีเซลล์เจริญไปเป็นพลาสมาเซลล์และสร้างแอนติบอดี ซึ่งวัตถุประสงค์ของงานวิจัยนี้ เพื่อศึกษาการแสดงออกในระดับยีนของนอทช์รีเซปเตอร์และนอทช์ลิแกนด์ในฟอลิคูลาเฮลเปอร์ทีเซลล์จากทอนซิลของมนุษย์ โดยวัดและคำนวนหาระดับการแสดงออกของ mRNA ของยีนต่างๆ ในฟอลิคูลาเฮลเปอร์ทีเซลล์(CD4+CXCR5hiICOShi) เทียบกับเฮลเปอร์ทีเซลล์กลุ่มนอนฟอลลิคูลา(CD4+CXCR5loICOSlo) ที่แยกได้มาจากต่อมทอนซิลของมนุษย์ ผลการศึกษาพบการแสดงออกในระดับยีนของนอทช์รีเซปเตอร์และนอทช์ลิแกนด์ในฟอลิคูลาเฮลเปอร์ทีเซลล์สูงกว่ากลุ่มเฮลเปอร์ทีเซลล์ที่ไม่จัดเป็นฟอลลิคูลาเฮลเปอร์ทีเซลล์

ABSTRACT

Notch signaling pathway is a highly conserved pathway for cell to cell communication and important for differentiation and functions of various cells. Follicular helper T cell (TFH) is one of the subsets of helper T cells that found mainly in secondary lymphoid organ. Their functions are to maintain and help B cell to differentiate into plasma cells and to produce antibody. The objective of this study is to investigate the expression profile of Notch receptors and theirs ligands in freshly isolated human tonsillar TFH. The relative mRNA expression was calculated from level of mRNA expression of TFH (CD4+CXCR5hiICOShi) comparing with non-TFH (CD4+CXCR5loICOSlo). The result revealed higher expression of Notch receptors and their ligands in TFH than non-TFH.

คำสำคัญ: นอทช์ ฟอลิคูลาร์เฮลเปอร์ทีเซลล์ ทอนซิล

Key Words: Notch, Follicular helper T cell, Tonsil

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Introduction

Notch signaling pathway is a highly conserved pathway for cell to cell communication. Its signaling is comprised of Notch receptors and Notch ligands. Notch is a type1 transmembrane protein present on cell surface. To date there are 4 different types of Notch receptors which are Noch1, Notch2, Notch3 and Notch4. Whereas Notch ligands comprised of 2 major classes which are Jagged (Jagged1 and Jagged2) and Delta (Dlk1, 3 and4).

The interaction between Notch receptor and their ligand from neighboring cells leads to signal sending to regulate function of cells. It regulates many cellular processes such as proliferation, differentiation, function, survival and also cell death (Robey et al.,2004).

Follicular helper T cell (TFH) is one of the CD4+ T helper cell subsets which mainly localize in secondary lymphoid tissues such as tonsils, lymph nodes and spleen. The transcriptional regulator of TFH differentiation is Bcl-6 (Nurieva et al., 2009). TFHs secrete IL-21 cytokine to help maintain the formation of germinal center (GC), and to regulate B cell differentiation by providing help to B cells. Previous study reported the unique transciptomic patterns of genes related to the Notch signaling pathway in human TFH. Notch1 and Hes1, a downstream target of the Notch signaling, were expressed at significantly higher level in TFH than other helper cell subsets (Inrun, 2011; Rasheed et al., 2006). Furthermore, T cell-specific deletion of Notch1 in mice exhibited impaired TFH development, correlating with deficient GC development and the absence of high affinity antibody (Auderset et al., 2013). From these results imply that Notch signaling is involved in TFH. So we want to investigate the expression of Notch in TFH.

Objective of the study

The aim of this study is to investigate the expression profile of Notch receptors and theirs ligands in freshly isolated human tonsillar TFH.

Methodology

Samples

Human tonsil tissues were collected from healthy individual who have hypertrophic tonsils and undergone an operation for tonsillectomy. All samples were obtained from King Chulalongkorn Memorial Hospital. This project was ethically approved from Institutional review board (IRB), faculty of Medicine, Chulalongkorn University. (IRB.No.539/55)

Isolation of TFH and nonTFH

Cells were isolated from freshly tonsil by mechanical disruption using blender. Mononuclear cells were collected by gradient density method using Ficoll-Hypaque. All cells were labeled with APC-anti-CD4, Alexa- Flour®488-anti-CXCR5 and PerCP-anti-CD278 (ICOS) antibodies (all of antibodies are mouse anti-human antibodies that were purchased from BD Biosciences, CA, USA), and sorted by Fluorescence activated cell sorting (FACS) using FACSAriaII (BD Biosciences CA, USA).

RNA extraction and cDNA preparation

Total RNA was extracted from freshly sorted TFH and nonTFH using QIAGEN RNeasy mini kit (Hilden, Germany).The reverse transcription of RNA to

cDNA was performed by using reagents of QIAGEN Quantitect® probePCR kit (Hilden, Germany). The RT-PCR program was set as following, 25°C for 10 min, 48 °C for 30 min and 95 °C for 5 min by PCRSprint (Thermo Scientific Hybaid, Rockford, U.S.A.).The cDNA were kept at -20°C.

Real-time PCR assay

Gene expression were measured by semi-quantitative RT- PCR using MJ miniOpticon (Bio-Rad, CA, U.S.A.). The relative expression level of each gene was normalized to that of a house keeping gene *GAPDH.* The relative mRNA expression was calculated from level of mRNA expression of TFH compared with non-TFH.

Result

Isolation of TFH and nonTFH from freshly dissected Tonsils

Population of mononuclear cells were gated as L1 on FSC and SSC. CD4+ T cells (L2) were selected from L1 and divided into 3 groups from expression marker ICOS and CXCR5 as show in Fig.1. TFH and non-TFH were isolated as population of CD4+ICOS^{hi}CXCR5^{hi} (L3) and CD4+ICOS^{lo}CXCR5^{lo} (L5) respectively. The expression of *Bcl-6* and *IL-21* mRNA were detected to confirm identity of sorted TFH (Fig. 2). TFH had higher expression of *Bcl-6* and *IL-21* than nonTFH. These results confirm phenotype of TFH.

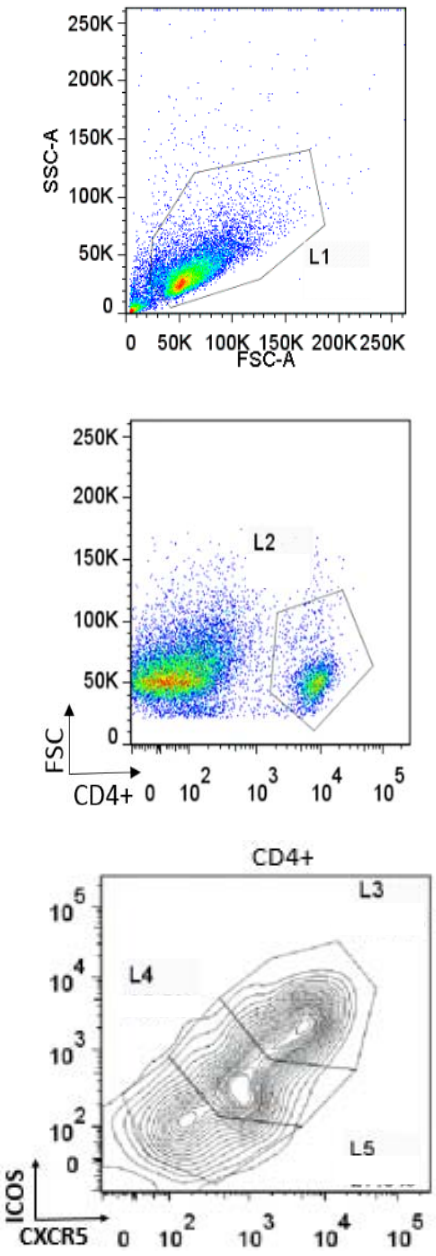

Figure 1 CD4+ T cells subset (L2) were separated from mononuclear cells (L1). TFH and nonTFH were identified in subset of CD4+ICOS^{hi}CXCR5^{hi} (L3) and CD4+ICOS^{lo}CXCR5^{lo} (L5) respectively

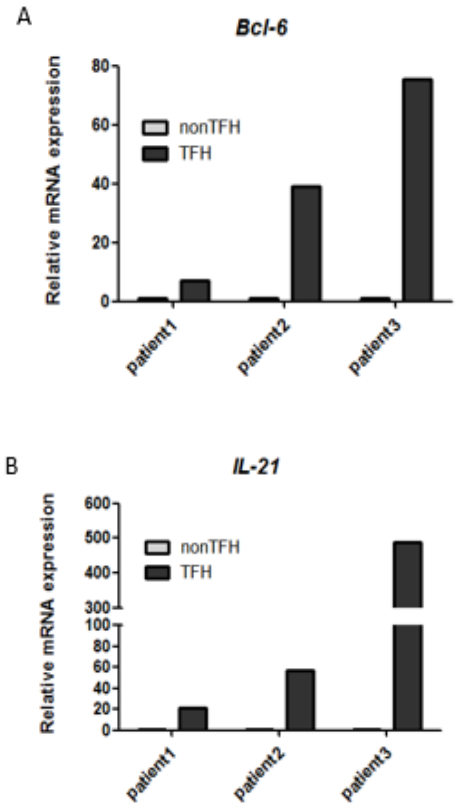

Figure 2 (A) *Bcl-6* and (B) *IL-21* mRNA expression were detected to confirm identity of freshly isolated human TFH. Each number represent as each patient (n=3)

Expression profiles of the Notch receptors and their ligands in freshly isolated TFH

Expression profiles of Notch receptors and Notch ligands in freshly isolated human TFH and non-TFH were detected by quantitative RT- PCR. Fold changes were calculated from the level of mRNA expression in TFH compared with non-TFH. *GAPDH* were used as internal control. The results show that patients had higher expression level of *Notch1* and *jagged2* than others notch receptors and ligands in TFH.

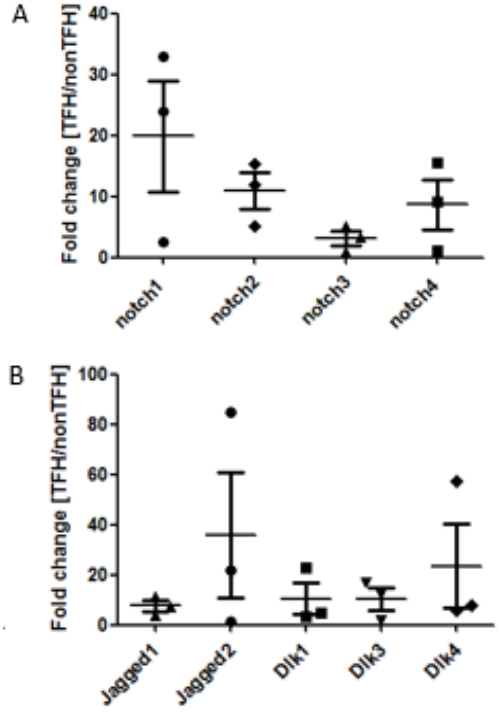

Figure 3 Expression profiles of (A) *Notch receptors* and (B) *ligands* in freshly isolated human TFH. Each point represent as each patient (n=3)

Discussion and Conclusion

TFH from freshly dissected tonsils can be separated from total lymphocytes using the expression of surface marker CD4+CXCR5^{hi}ICOS^{hi}. From the semi-quantitative RT-PCR results show that TFH had higher expression of both *Bcl6* and *IL-21* than non-TFH. These results confirmed the identity of TFH. The tendency of *Notch1* and *jagged2* were higher in TFH, compared with non-TFH. These results imply that Notch signaling is involved in TFH differentiation and/or function but the detailed mechanism need to be studied in the future. However, the levels of expression varied widely among patients. It may be the result of

low sample numbers of patients, differences in genetic backgrounds, age, gender, including pathogen exposure history of each patient.

Acknowledgment

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