学位論文（要約）

Studies of murine NK-triggering receptors expressed on myeloid cells and their response to Hepatitis B virus infection

(マウス骨髄細胞での NK 関連レセプターの発現と HBV 感染応答に関する研究)

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Type I interferons (Type I IFNs) can activate natural killers cell and macrophages contribute to the clearance of pathogens. They can also up regulate antigen presentation to increase the host defense, thus play an important role in immune response. Paired receptor refers to a cluster of membrane proteins characterized by similar extracellular regions but different transmembrane and cytoplasmic regions. By having the activating receptor associated by ITAM-containing adaptor, such as DAP12 or ITIM containing inhibitory receptors. Paired receptors can give activating or inhibitory signals, which tightly regulate the immune system and dysregulation of this signals often causes autoimmunity, allergy, and various infectious diseases. Triggering receptors expressed on myeloid cells (Trem) proteins are a family of cell surface receptors expressed broadly on myeloid cells。

In this study, we have identified Trem5, Treml6 and pDC-Trem are typical paired receptors and murine specific. The mRNA expression of pDC-Trem and Treml6 is induced by polyI:C in a type I IFN dependent manner, which secondarily through mainly MDA5/MAVS pathways rather than TICAM-1 pathway in BMDCs. Moreover, the tissue distributions show that these paired Trem genes highly expressed in spleen, which infer their potential roles acting in immune responses.

The counterbalance theory, proposed by Barclay and Hatherley, is used to describe the evolution of paired receptors. In this theory, pathogens target the inhibitory receptors, probably by binding to the activating receptors because of its similar extracellular regions. Thus, the activating receptors possibly evolved from the inhibitory receptors. Phylogenetic analysis shows that murine paired Trem genes were related most closely to Treml7 genes, which suggested that murine paired Trem genes and Treml7 originate from the same ancestral gene. Here we deduced the evolution of murine paired Trem genes. First, the common ancestral gene encored two ITIMs in its CPR. After brunching to murine linage, ancestral paired Trem gene containing ITIMs were duplicated, and the one of them evolved murine Treml6 gene. Another duplicated gene was translocated between Trem1 and Foxp4 genes and lost ITIMs. Finally, the common ancestor of Trem5 and pDC-Trem genes was duplicated, and evolved murine Trem5 and pDC-Trem. The deduced evolutionary model suggests that activating Trem5 and pDC-Trem originated from inhibitory ITIM-containing Trem gene, which strongly support this counterbalance theory.

Trem5 and pDC-Trem is associated with DAP12 for post-translational modification and cell surface expression, lead to activation of Src-family kinases and subsequent

phosphorylation of tyrosine residues in the ITAMs. However, although both Trem5 and pDC-Trem are DAP12 associated activating receptors, polyI:C failed to stimulate Trem5 mRNA in BMDCs and spleen, whereas pDC-Trem mRNA expression was induced by polyI:C , which dependent on type I interferon via MAVS pathway. Therefore, the expression of Trem5 and pDC-Trem genes are differentially regulated by different PRRs.

DAP12 axis, associated with paired receptors, can have not only activation but inhibitory roles. pDC-Trem formed a complex with Plexin A1 and DAP12 , arise positive signal for type I IFN production in pDCs. However, Trem2 and DAP12 axis act as the negative regulators of cytokine production including type I IFN, IL-6 and IL-12 in BMDCs. Moreover, Trem1 activation dampened LPS induced IL-12 family member cytokines in vivo. On the other side, ITIM-containing receptors also function as activation or inhibitory role in a cell type-dependent manner. Ly49Q enhances TLR9-mediated signaling events, positively regulates type I IFN in an ITIM-dependent manner. On the contrast, ITIM-containing DC immunoreceptor (DCIR) on human monocyte-derived DCs inhibits TLR8-mediated IL-12 and TNF-α production. Similarly, DCIR on pDCs inhibits TLR9-induced IFN-alpha production while leaving up-regulation of costimulatory molecule expression unaffected. In our results, both Treml6 and pDC-Trem expression were induced BMDCs and splenic DC subsets, followed by type I IFN pathway. Therefore, it is possible that Treml6 and pDC-Trem act as a positive or negative regulator of proinflammatory cytokines in DC subset.

Our report is the first founding of typical paired receptors in Trem gene clusters. Interestingly, although both of Trem5 and pDC-Trem is associated with DAP12, the expression pattern varies each other in DC subsets. Whether paired Trem protein act as activation or inhibitory role is remained unclear, further studies will uncover the function of paired receptors in DC subsets.

Hepatitis B virus infection induce chronic inflammatory liver injury , causes liver cirrhosis and the development hepatocellular carcinoma(HCC).Immune responses are triggered in NK cells fight against hepatitis B infection, both cytokines and cytolysis function of NK cells are involved in the clearance of HBV. IL15, a main cytokine for NK cell maturation and activation, can suppress HBV infection. Besides, DC-activated NK cells induce massive HBV-infected hepatocyte degeneration through the Fas/FasL system, and granzyme H is essential for HBV eradication, by degrading the one of HBV protein-HBx protein (HBx). On the other side, HBV infection causes aberrations in NK cells function, the pro-inflammatory molecules of NK cells are disrupted in long-term hepatitis B infection.

Here, we set up an in vitro co-culture system to study the role of HBV on NK cells during the crosstalk with macrophages followed by PolyI:C stimulation model. We found the function of NK cells, upon the interaction of macrophages to produce the IFN-γ, is enhanced upon exposure to HBV. Furthermore, our data shows that HBV induced IFN-γ was not mainly dependent on the cell to cell contact of HBV infected hepatocytes and NK cells/macrophages, which suggest HBV-infected hepatocytes secreted some unknown factors which enhance the crosstalk of NK cell and macrophages by producing more IFN-γ.

It has been reported that HBV impaired the NK cells functions in human cells model, but NK cells themselves are not directly modulated by HBV, one of the cell type, pDC, maybe participate in the progress of HBV induced NK cell mal-function. HBV can interfere with the functional interaction between NK cell and pDCs, which significantly reduce pDC-induced IFN-γ by NK cells. However, HBV did not affect the cytolytic of NK cells. Further research shows that HBV interfered with TLR-9 induced pDC function, which result in the inhibition of cytokine production.

On the contrast of the inhibition function of HBV in pDC and NK cells crosstalk, we found some unknown factors, secreted by HBV infected hepatocytes, activate NK cell and macrophage interaction. It is well known that nucleic acid sensing of virus is the beginning of antiviral immune responses. HBV genome is consist of a double-stranded 3.2kb DNA, but during the life cycle of HBV in hepatocytes, its covalently closed circular DNA (cccDNA) is transcribed to generate four RNA species: the 3.5, 2.4, 2.1 and 0.7kb viral RNA transcripts. These transcripts can be recognized by PRRs, and trigger the innate immune response such as the production of IFNs, to the clearance of HBV. In the recent report, Takaoka's group have found the retinoic acid-inducible gene-I (RIG-I) is a key PRR that participate in HBV-induced Type III IFN in Hepatocytes. Moreover, the only 3.5kb transcript, which is named pgRNA, has the potential role of elicit the Type III IFN. Finally, they identified the 5'-end of HBV pgRNA, called epsilon (ε), interact with RIG-1 and exert the antiviral activity. However, our data show that the Type II interferon (IFN-γ), but not III, is induced by HBV in the crosstalk of NK cells and macrophages. Whether the pgRNA of HBV and related PRRs participate in the production of IFN-γ or not is still remain unclear.