学位論文内容の要旨
（Summary of dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名　鄧孟垚
（Degree conferred: Doctor of Philosophy）　（Name of recipient:　Mengyao Deng ）

学 位 論 文 題 名
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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 ）

Background and objectives

Some members of multiple immune receptors, such as killer inhibitory receptorw (KIRs), have extremely high homology in their extracellular domain. These receptors are called paired receptors that are generally associated by immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor, and immunoreceptor tyrosine-based inhibition motif (ITIM)-containing inhibitory receptors. Since the exist and the function of paired receptors expressed on myeloid cells are poorly understood, we want to understand paired receptors in myeloid cells is important to regulate immune system by the immunotherapy against cancers and pathogens.

Hepatitis B virus, is one of the most dangerous and prevalent infectious agents that lead to liver disease in humans, NK cells play important roles in the induction of immune responses to eradicate HBV infection from the infected liver, however, NK cell function is disrupted with prolonged HBV infection. It is well known that mDC and macrophages (Mf), can activate NK cell by DC-NK or Mf-NK crosstalk, here, we want to understand the role of HBV infection to mDC/Mf induced NK cell activation.

Materials and methods

Cell culture, Database analysis, Gene cloning, quantitative real-time PCR, ELISA, Immunoblotting and immunoprecipitation, Flow Cytometric Analysis, cell co-culture.

Results

We found murine Trem5, Trem-like transcript-6 (Treml6) and pDC-Trem genes are typical paired receptors. These receptors are originated from from inhibitory Trem genes and murine specific.Trem5 and PDC-Trem is directly associate with DAP12 and require it for their expression on cell surface,but not Treml6. Type I IFN secondary to poly I:C stimulation is required for the expression of Treml6 and pDC-Trem genes on myeloid cells

Mouse hepatocytes, which infected by Hepatitis B virus, can participate in NK cells and macrophage crosstalk, by the enhanced level of Interferon gamma and the following NK cell activation.

Discussion

Triggering receptors expressed on myeloid cells (Trem) proteins that are a family of cell surface receptors to control innate immune responses such as proinflammatory cytokine production. In my doctor thesis, I report that murine Trem5, Trem-like transcript-6 (Treml6) and pDC-Trem (as known as Trem4) genes are typical paired receptors. The term of “paired receptor”, such as NK receptors, is commonly used to describe families of membrane receptors that have very similar extracellular

regions but different transmembrane and cytoplasmic regions. Activating receptors including Trem5 and pDC-Trem associate with immunoreceptor tyrosine-based activation motif (ITAM)-containing DAP12 in transmembrane region, whereas inhibitory receptor including Treml6 encode immunoreceptor tyrosine-based inhibition motif (ITIM) in cytoplasmic region. Our study is the first report of the typical paired receptors in Trem family. We also elucidate that these paired Trem genes originated from inhibitory receptors by comparative genomic and phylogenetic analysis. Our data clearly support the "counterbalance theory", which hypothesize that activating receptors evolve from inhibitory receptors.

The function of paired receptors expressed on myeloid cells including dendritic cell (DC) subsets is poorly understood. We also find that type I interferon secondary to polyinosinic-polycytidylic acid (polyI:C) stimulation is required for the expression of Treml6 and pDC-Trem genes for conventional DCs and plasmacytoid DCs, whereas Toll-like receptor agonists including polyI:C failed to induce the expression of Trem5 gene. Therefore, our finding indicates Trem5 and pDC-Trem genes are differentially regulated in DC subsets. Further studies of these paired Trem proteins will uncover the functions 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. Recently research found that HBV can suppress NK cells by modifying plasmacytoid dendritic cells (pDcs), by disrupting the production of IFN-γ produced by pDCs, thus hamper pDCs-NK cells crosstalk. Interestingly, our results show that Hepatitis B virus infection can enhance IFN-γ produced by NK cell and macrophages crosstalk ,under a poly I：C stimulation model. Moreover, we also found that soluble factors secreted by HBV-infected hepatocyte, participate in this progress. Further studies will be focus on which soluble factors are necessary for this progress.

Conclusion

Our data is the first report of typical paired receptors of Trem genes, and the data indicate that unique Trem receptors were evolved in mice and DAP12-associated Trem5 and pDC-Trem genes are differentially regulated in DC subsets.

In vitro cell co-culture experiment, we found that Hepatitis B virus is involved in NK-macrophage crosstalk by upregulating the production of IFN-Y, which may activate NK cells and leading the clearance of this virus. This reaction is mediated by soluble factors secreted by hepatocytes under the infection of Hepatitis B virus. Further studies will be focus on which soluble factors are necessary for this progress.