論　文　概　要

MafB is the primary regulator of complement component C1q

(MafB は補体 C1q のマスターレギュレーターである)

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Abstract

Background and purpose

Transcription factor MafB belongs to the large Maf family that regulates target genes by binding into Maf recognition element (MARE) in the promoter region. In hematopoietic system, MafB is specifically expressed in monocytes and macrophages, and affects proliferation and differentiation of macrophages. Previous studies suggested MafB functions in physiology of macrophages and may play a role in macrophage related-diseases.

C1q, the first subcomponent of the classical complement pathway, is the receptor of apoptotic cells involved in efferocytosis of macrophages. The deficiency of C1q in human and mouse macrophages leads to the development of systemic lupus erythematosus (SLE)-like disease because of the failure of efferocytosis. However, molecular mechanism of *C1q* regulation is largely unknown.

Nuclear receptors are transcription factors that are involved in the metabolic and immune activities of macrophages. Liver X receptors (LXR) promotes the apoptotic cell clearance to suppress inflammatory pathways. Deficiency of RXRα, PPARγ or PPARδ induced autoimmune kidney diseases. LXR/RXR has been shown to directly regulate the expression of *Mafb* in macrophages. However, there is no report on MafB function in efferocytosis and development of autoimmune disease.

In this study, I aimed to clarify the role of MafB in efferocytosis and its connection with autoimmune diseases by using *Mafb*-deficient mice.

Materials and methods

To demonstrate whether MafB deficient mice develop autoimmune diseases, I analyzed the serum levels of auto-antibodies and the accumulation of immune complex in glomeruli of kidney. Then, I evaluated glomerulonephritis and the urine protein. Next, I evaluated the phagocytosis of *Mafb*-deficient macrophages using CellTracker or pHrodo-labelled apoptotic cells. To examine the regulation of *C1q* by MafB, I checked the expression of C1q in WT or *Mafb*$^{-/-}$ macrophages using qPCR, Western blot analysis, and hemolysis assay; and performed promoter analysis. To clarify whether MafB promotes the efferocytosis via *C1q* regulation, I rescued the efferocytosis of *Mafb*-deficient macrophages by adding the serum containing C1q protein and checking phagocytosis by FACS analysis. To clarify whether PPARδ is upstream regulator of MafB, I induced PPARδ expression in macrophages to check expression of *Mafb*, and then performed promoter analysis. To determine whether MafB was involved in PPARδ - C1q axis, I induced PPARδ in WT and *Mafb*$^{-/-}$ macrophage and checked *C1q* expression.

Results

Here I found that *Mafb*-deficient mice developed autoimmune diseases more seriously than WT mice, due to the increase of serum levels of auto-antibodies and glomerulonephritis, and the higher urine protein level. The *Mafb*$^{-/-}$ macrophages failed to engulf or bind to fluorescent apoptotic cells when I fed fluorescent apoptotic cells to macrophages *in*

vitro and *in vivo*. These evidences indicated that MafB affects autoimmune diseases through efferocytosis process.

I investigated whether C1q, composed of C1qa, C1qb, and C1qc, was the target molecule of MafB in efferocytosis process. My result showed that the expression of *C1q* in macrophages was decreased. C1q levels and C1q activity in the classical complement pathway of *Mafb*$^{-/-}$ mice were reduced. Moreover, the expressions of human *C1Q* genes were reduced in *MAFB*-knock down human macrophage THP-1 cells. Promoter analysis data also showed in both humans and mice, MafB directly regulates *C1q* genes through the half-MARE sites. The efferocytosis of *Mafb*-deficient mice was rescued by using WT serum containing C1q protein, suggested the disruption of efferocytosis in *Mafb*$^{-/-}$ macrophages is caused by reduction of C1q.

Moreover, I found that PPARδ is the upstream regulator of MafB in efferocytosis. One paper showed PPARδ directly regulates *C1q*. In *Mafb*-deficient macrophages, the expression of *C1q* under PPARδ induction was lower than in WT macrophages, indicated MafB is important for the basal expression of *C1q* genes.

Discussion

For the first time, I demonstrated that MafB is an important factor for the expression of *C1qa*, *C1qb*, and *C1qc*, which assemble the C1q protein. C1q plays an important role in the development of autoimmune diseases. Clinical reports have shown that 90% of C1q-deficient patients have

autoimmune diseases. Consistent with these reports, I have observed a similar phenotype in *Mafb* deficient mice. Therefore, it is likely that MafB also regulates *C1q* genes in other species. The knock-down of MAFB in human macrophages causes the down-regulation of *C1Q* genes. Moreover, in zebrafish, *C1q* promoters have half MAREs, and the preliminary result of a zebrafish *Mafb* morpholino oligo injection showed a reduction in zebrafish *C1q* gene expression (data not shown). The origin of the classical pathway was found in sea lamprey and I also found a half-MARE sequence from the 5' upstream of lamprey C1q-like genes. Therefore, the MafB binding site may contribute to evolution from the primitive lectin pathway to the classical pathway that supports adaptive immunity.

Conclusion

The present results indicate that MafB is the primary regulator of *C1q* genes. In addition, MafB has a pivotal role in the efferocytosis and inhibition of autoimmune disease through the regulation of *C1q* genes.