Sema4D Deficiency Improved Metabolic Phenotype through Immature B Cell Development in Diet-induced Obesity

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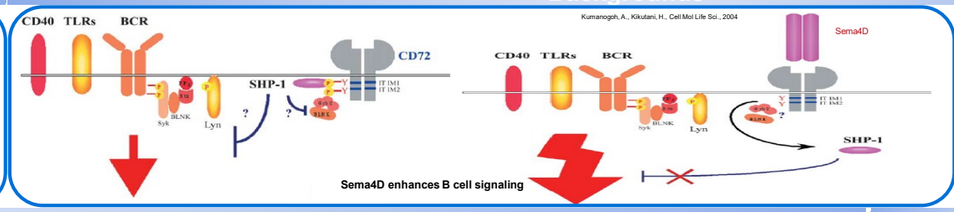
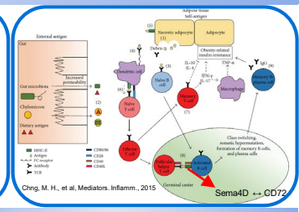
Abstract

Recent studies have been proposing the potential that B cells exacerbate insulin resistance. However, it is still not clearly understood which molecules or mechanisms are underlying the possibility. We found that Sema4D deficiency inhibited B cell differentiation and this decreased adipose tissue inflammation in diet-induced obesity. High-fat diet (HFD)-induced obese Sema4D knockout mice exhibited ameliorated systemic inflammation and produced lower levels of pathogenic immunoglobulin IgG2c. These led to decreased infiltration of macrophages into adipose tissues and inhibited macrophage switch to pro-inflammatory states. Reduction of adipose tissue macrophages alleviated adipose tissue inflammation. Relieved adipose tissue inflammation improved insulin resistance and HFD-induced obesity, normalized adipose tissue homeostasis, and lessened WAT mass. This study suggests the importance of Sema4D and B cells in developing insulin resistance and a potential therapeutic target for regulating metabolic homeostasis.

Backgrounds





Results

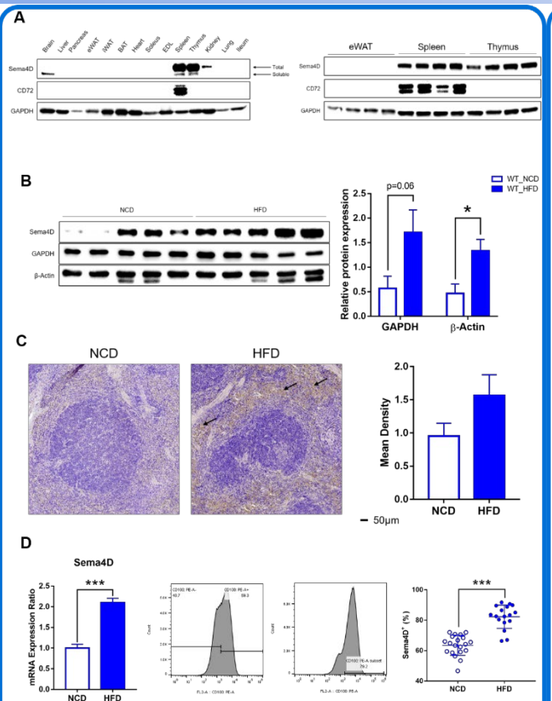


Figure 1. Sema4D expression is increased in the spleens of HFD WT compared to NCD WT. (A) Protein expression pattern of Sema4D and CD72 in various tissues from 8-week-old C57BL/6N and protein expression of Sema4D and CD72 in epididymal adipose tissue, spleen, and thymus from 8 weeks old C57BL/6N mice. (B) Protein expression of Sema4D (left) and relative expression level of Sema4D to GAPDH and β-actin (right). (C) Immunohistochemical analysis of Sema4D in WT NCD and HFD spleen (arrow). (D) Relative mRNA level of Sema4D in splenocytes (left) and flow cytometry analysis of Sema4D in WT NCD and HFD splenocytes (right). Representative images from FlowJo (middle). Data are the means ± standard error of means (SEM); *P < 0.05, **P < 0.01, ***P < 0.001.

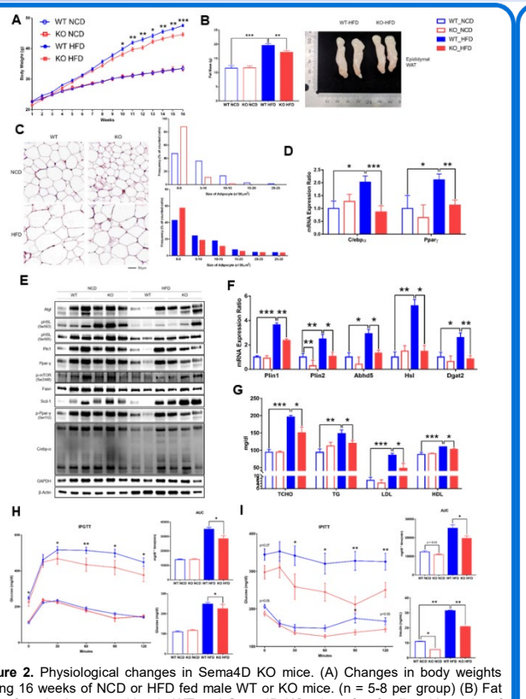


Figure 2. Physiological changes in Sema4D KO mice. (A) Changes in body weights during 16 weeks of NCD or HFD fed male WT or KO mice. (n = 5-8 per group) (B) Fat mass from body-composition in WT and Sema4D KO mice after feeding 16 weeks of diet (left) and representative image of epididymal white adipose tissue (right). (C) Representative images of H&E staining in epididymal WAT in NCD and HFD Sema4D KO (left) and distribution of adipocyte sizes (right). (D) Relative mRNA levels of genes related to adipogenesis in eWAT were measured by qPCR; the values were normalized to 36B4 (E) Activation of lipolysis genes were assessed by immunoblotting. (F) Relative mRNA levels of genes related to lipid metabolism in eWAT were measured by qPCR; the values were normalized to 36B4. (G) Serum total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol. (H) Glucose tolerance test of WT or KO mice after 16-week NCD or HFD challenge (left), AUC of the test and fasting blood glucose levels (right). (I) Insulin tolerance test of WT or KO mice after 16-week NCD or HFD challenge (left), AUC of the test and fasting blood insulin levels (right).

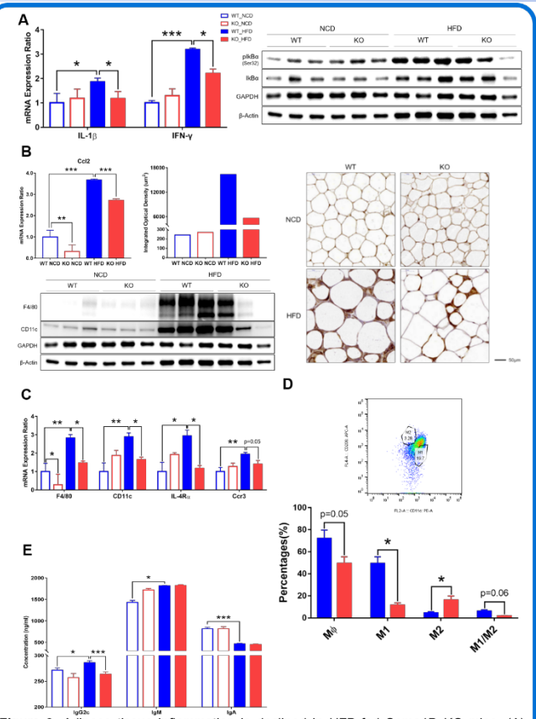


Figure 3. Adipose tissue inflammation is declined in HFD-fed Sema4D KO mice. (A) qPCR analysis of IL-1β and IFN-γ in eWAT of WT or KO mice challenged 16-week NCD or HFD; expression was normalized to that of 36B4 and protein level of phosphorylated IκB-α was analyzed by immunoblotting in eWAT. (B) The mRNA expression of CCL2 (left), immunohistochemical analysis of crown-like structures surrounding adipocytes in Sema4D WT and KO given NCD and HFD; paraffin sectioned eWAT samples were stained with F4/80 (middle) and integrated optical density of F4/80 was measured (right). (C) Protein expression levels of F4/80 and CD11c were analyzed in eWAT and the mRNA expression levels of pro-inflammatroy genes in eWAT SVF were analyzed. (D) Flow cytometry analysis of macrophages in eWAT SVF. (E) Concentrations of antibody subtypes in mice eWAT lysates.

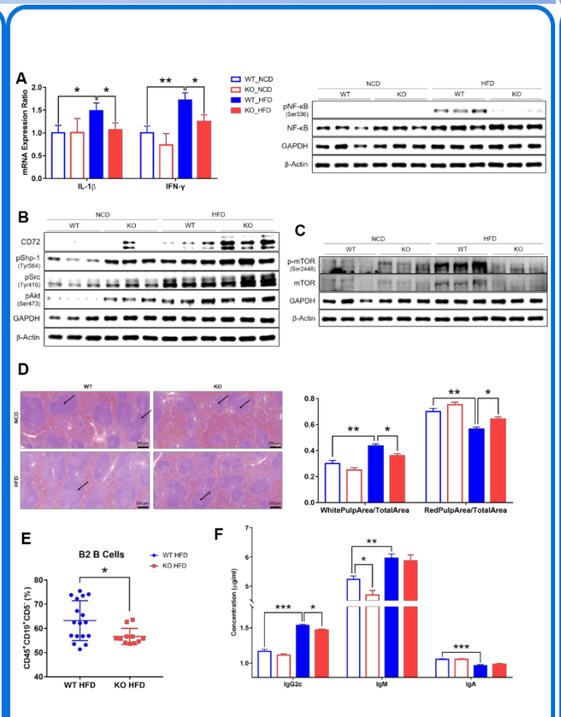
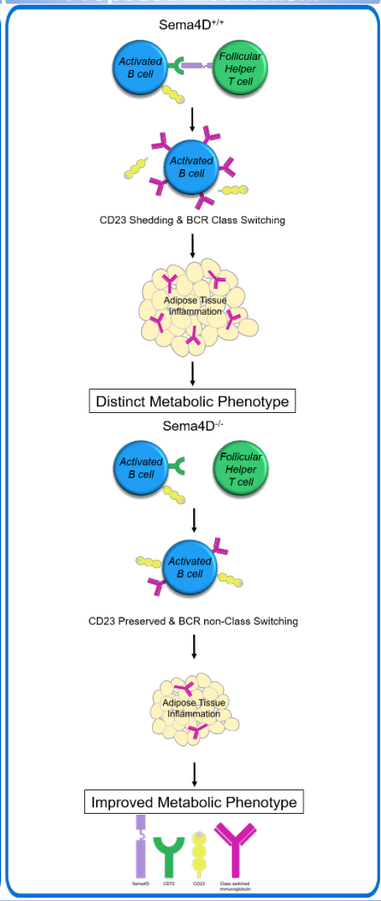


Figure 4. B cell responses is decreased in the spleen of HFD-fed Sema4D KO mice. (A) qPCR analysis of IL-1β and IFN-γ in spleen of WT or KO mice on 16-week NCD or HFD; expression was normalized to that of 36B4 and phosphorylated NF-κB p65 subunit level was assessed by immunoblotting in spleen. (B) Inhibition of CD72 signaling pathway was analyzed by immunoblotting in the spleens (C) Immunoblotting analyzation of mTORC1 activation and mTOR activity in the spleens. (D) Representative H&E staining images of NCD and HFD Sema4D WT and KO spleens (arrow: white pulp) (left) and white pulp area per total area and red pulp area per total area (right). (E) Flow cytometry analysis of B2 B cells (CD45+CD19+CD5-). (F) IgG2c, IgM and IgA concentrations in mice serum.

Proposed Mechanism



Conclusions

- Sema4D expression increased in the spleens from high-fat diet-induced obese mice
- Sema4D knockout mice had decreased bodyweights, lessened WAT masses and adipocyte sizes, normalized adipose tissues homeostasis, and alleviated glucose and insulin intolerance
- Adipose tissue inflammation was ameliorated in obese Sema4D knockout mice
- Adipose tissue macrophages were decreased and the macrophages tended to be less pro-inflammatory in obese Sema4D knockout mice for the decline of IgG2c
- Systemic inflammation was relieved, so the production of pathogenic immunoglobulins was inhibited in the spleens form Sema4D knockout mice

⇒ Sema4D is a potent therapeutic target molecule that regulates B cells-induced insulin resistance