GPNMB plays a protective role against obesity-related metabolic disorders by reducing macrophage inflammatory capacity

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学 位 論 文 の 内 容 要 旨

GPNMB plays a protective role against obesity-related metabolic disorders by reducing macrophage inflammatory capacity

GPNMB はマクロファージの炎症性を低下させ、肥満関連代謝異常を軽減する。

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A. Background

Obesity is defined as abnormal fat accumulation, which impairs metabolic health. Currently, obesity is a public health problem affecting millions of people worldwide and its prevalence has nearly tripled since 1975. Obesity is also known as a risk factor for various diseases such as cardiovascular diseases, diabetes, musculoskeletal disorders, and some cancers.

Obesity has been associated with metabolic disorders due to chronic low-grade inflammation in white adipose tissue (WAT). During pathological expansion, macrophages are recruited into WAT, and these recruited inflammatory macrophages are considered as the main contributors in WAT inflammation, leading to metabolic disorders associated with obesity. Therefore, reducing WAT inflammation by regulating macrophage inflammatory capacity is an attracting therapeutic strategy for the treatment of obesity-related metabolic disorders.

We identify glycoprotein nonmetastatic melanoma protein B (GPNMB) as a gene that is highly expressed in the WAT of obese mice. It has been reported that GPNMB is associated with inflammation in several organs and lipogenesis in liver. However, a role of GPNMB in obesity-related metabolic disorders remains controversial, and needs to be elucidated. Here we explore the role of GPNMB in obesity-related metabolic disorders using mice with target deletion of GPNMB.

B. Methods

For in vivo studies, GPNMB-deficient (GPNMB-KO) mice were generated (C57BL6N background). GPNMB-KO and wild-type (WT) mice at the age of 10 weeks old mice were fed with high-fat diet (HFD) for up to 14 weeks to induce obesity and insulin resistance, while body weight was measured every week. For macrophage depletion, clodronate liposomes were intraperitoneally injected into the mice once in 2 weeks during HFD-feeding. Systemic metabolic functions were assessed by insulin tolerance test (ITT) and intraperitoneal glucose tolerance test (ipGTT) in mice fed an HFD for 12 weeks, then WAT was extracted for histological analysis, and RNA and protein extraction.

For in vitro studies, thioglycolate-elicited peritoneal macrophages (TEPMs) were isolated from GPNMB-KO and WT mice. Inflammatory phenotypes of

TEPMs were assessed in the presence or absence of recombinant soluble GPNMB extracellular domain (ECD) treatment. Anti-CD44 antibody and high molecular weight hyaluronic acid treatments were given to TEPMs to block GPNMB-ECD binding to CD44. For exploring a role of NF-κB in the mechanisms underlying the GPNMB anti-inflammatory functions, phosphorylation and nuclear translocation of NF-κB was assessed in TEPMs treated with TNF-α.

C. Results

1. GPNMB expression is increased in WAT of obese mice

We identified GPNMB as a gene expressed in the WAT of obese mice using signal sequence trap method. Subsequently, we found that GPNMB was highly expressed in the WAT of obese mice comparing to that in lean mice at both RNA and protein levels. In WAT of lean mice, stromal vascular fractions, especially macrophages, showed dominant expression of GPNMB. In contrast, GPNMB expression was predominant in mature adipocytes in WAT of obese mice, although its expression was also increased in SVF comparing to that in lean mice. In addition, we found that peritoneal macrophages highly expressed GPNMB. GPNMB expression was reduced by pro-inflammatory stimuli such as TNF-α and LPS, while anti-inflammatory cytokine, IL-10, increased GPNMB expression in peritoneal macrophages. These data strongly suggest that GPNMB plays an important role in the WAT dysfunction during obesity.

2. Loss of GPNMB aggravates obesity-related metabolic disorders

During the HFD feeding, body weight was similar in WT and GPNMB-KO mice. Nevertheless, GPNMB-KO mice exhibited worsened insulin resistance, despite the obesity and adiposity similar to WT mice. Histological analysis showed increase in crown-like structures and macrophage infiltration in the WAT of GPNMB-KO mice comparing to those in WT mice fed with HFD. We further identified that increased macrophages in the WAT were mostly CD11c-positive recruited macrophages. Consistently, remarkable increase in inflammatory cytokines expression was detected in the the WAT of obese GPNMB-KO mice. These data collectively indicate that loss of GPNMB aggravates WAT inflammation, and consequently worsened the metabolic disorders associated with obesity.

3. GPNMB regulates inflammatory capability in macrophages

Thioglycolate-elicited peritoneal macrophages (TEPMs) isolated from GPNMB-KO mice showed enhanced inflammatory genes expressions comparing to those in TEPMs isolated from WT mice. Of note, supplementation of recombinant soluble GPNMB-ECD abolished the enhanced inflammatory capacity in TEPMs isolated from GPNMB-KO mice. When treated with conditioned medium derived from GPNMB-KO TEPMs, 3T3-L1 adipocytes showed increased inflammatory genes expressions and decreased insulin sensitivity, while conditioned medium derived from GPNMB-KO TEPMs treated with recombinant GPNMB-ECD did not show such effects. These data suggest that GPNMB ameliorates WAT inflammation by reducing macrophage inflammatory capacity.

4. GPNMB negatively regulates inflammatory capability in macrophages via CD44-NF-κB pathway

GPNMB has been reported to interact with CD44, and we confirmed the biding of GPNMB-ECD to CD44 receptor in RAW 264.7 macrophages. Furthermore, blocking the interaction with CD44 using CD44 antibody or high molecular weight hyaluronic acid suppressed the enhanced inflammatory capacity in TEPMs isolated from GPNMB-KO mice. We also found that NF-κB activation and nuclear translocation in response to inflammatory stimuli were enhanced in TEPMs isolated from GPNMB-KO mice. Supplementation of GPNMB-ECD or anti-CD44 antibody abolished the enhanced NF-κB signaling in GPNMB-KO TEPMs. These data strongly suggest that GPNMB negatively regulates the inflammatory capacities in macrophages by blocking the CD44-NF-κB pathway.

5. Macrophage dysfunction plays a central role in aggravated obesity-related metabolic disorders in GPNMB-KO mice

To explore the role of macrophages in the worsened obesity-related metabolic disorders in GPNMB-KO mice, we depleted macrophages in mice using clodronate

liposomes. Administration of clodronate liposomes caused temporal decrease in body weight; however, the body weight of mice became similar in both WT and GPNMB-KO mice of vehicle- and clodronate-injection group after 12 weeks of HFD. Of note, macrophage depletion by clodronate liposomes completely abolished the aggravated obesity-related metabolic disorders in GPNMB-KO mice. Histological analysis showed less crown-like structures and decrease in macrophage infiltration, especially for CD11c-positive recruited macrophages, in the WAT of clodronate liposome injected mice, and macrophage infiltration in the WAT became similar in WT and GPNMB-KO mice after clodronate injection. Consistently, inflammatory cytokines expressions were reduced, and their enhanced expressions in the WAT of GPNMB-KO mice were disappeared after clodronate injection. These data indicate that loss of GPNMB causes aggravated obesity-related metabolic disorders largely through dysregulated macrophages in the WAT.

D. Discussion

In this study, we revealed that GPNMB, which is abundantly expressed in hypertrophied adipocytes and macrophages, plays a critical role in the WAT inflammation and metabolic disorders in obesity. The role of GPNMB in obesity and its-related metabolic disorders remained controversial because of the inconsistent results reported in previous papers. By using the GPNMB-KO mice, we here revealed that GPNMB plays a protective role against obesity-related metabolic disorders, while it showed no effect on obesity and adiposity.

The main limitation of this study is that we used systemic null knockout mice to investigate the role of GPNMB. Therefore, the differential contribution of GPNMB expressed in specific cells, such as adipocytes, macrophages, or hepatocytes remains unclear. Experiments using tissue-specific conditional GPNMB knockout mice are needed to further elucidate a role of GPNMB in obesity.

In the current study, we showed that GPNMB-ECD binds to CD44 in macrophages, and blocks CD44-NF-κB pathway in macrophages. However, detailed mechanisms by which GPNMB binding affects CD44 function, and possible other receptors for GPNMB in macrophages besides CD44 remain to be elucidated.

Our data reveal the protective role of GPNMB against obesity-related metabolic disorders through reducing macrophage inflammatory capability; therefore, GPNMB activation has a therapeutic potential for the treatment of obesity-related metabolic disorders.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第3138号	氏　名	ADAM PRABATA
論文題目 Title of Dissertation	GPNMB plays a protective role against obesity-related metabolic disorders by reducing macrophage inflammatory capacity GPNMB はマクロファージの炎症性を低下させ、肥満関連代謝異常を軽減する。		
審査委員 Examiner	主　査 Chief Examiner 小川 渉 副　査 Vice-examiner 児玉裕三 副　査 Vice-examiner 古屋敷智之		

（要旨は１，０００字～２，０００字程度）

In this study, the candidate has found that glycoprotein nonmetastatic melanoma protein B (GPNMB) is highly expressed in the WAT of obese mice, and investigated the role of GPNMB in obesity-related metabolic disorders using mice with target deletion of GPNMB.

The candidate identified GPNMB as a gene expressed in the WAT of obese mice using signal sequence trap method, and found that GPNMB was highly expressed in the WAT of obese mice comparing to that in lean mice. In WAT of lean mice, stromal vascular fractions, especially macrophages, showed dominant expression of GPNMB, and GPNMB expression was predominant in mature adipocytes in WAT of obese mice, although its expression was also increased in SVF comparing to that in lean mice. The candidate found that peritoneal macrophages highly expressed GPNMB. GPNMB expression was reduced by pro-inflammatory stimuli such as TNF- and LPS, while anti-inflammatory cytokine, IL-10, increased GPNMB expression in peritoneal macrophages. After feeding with HFD, GPNMB-KO mice worsened insulin resistance, despite the obesity and adiposity similar to WT mice. Histological analysis showed increase in crown-like structures and macrophage infiltration in the WAT of GPNMB-KO mice comparing to those in WT mice fed with HFD. Increased macrophages in the WAT were mostly CD11c-positive recruited macrophages. Thioglycolate-elicited peritoneal macrophages (TEPMs) isolated from GPNMB-KO mice showed enhanced inflammatory genes expressions comparing to those in TEPMs isolated from WT mice. Supplementation of recombinant soluble GPNMB-ECD abolished the enhanced inflammatory capacity in TEPMs isolated from GPNMB-KO mice. When treated with conditioned medium derived from GPNMB-KO TEPMs, 3T3-L1 adipocytes showed increased inflammatory genes expressions and decreased insulin sensitivity, while conditioned medium derived from GPNMB-KO TEPMs treated with recombinant GPNMB-ECD did not show such effects. The candidate confirmed the biding of GPNMB-ECD to CD44 receptor in RAW 264.7 macrophages. Blocking the interaction with CD44 using CD44 antibody or high molecular weight hyaluronic acid suppressed the enhanced inflammatory capacity in TEPMs isolated from GPNMB-KO mice. NF-kB activation and nuclear translocation in response to inflammatory stimuli were enhanced in TEPMs isolated from GPNMB-KO mice. Supplementation of GPNMB-ECD or anti-CD44 antibody abolished the enhanced NF-kB signaling in GPNMB-KO TEPMs. To explore the role of macrophages in the worsened obesity-related metabolic disorders in GPNMB-KO mice, the candidate depleted macrophages in mice using clodronate liposomes. Administration of clodronate liposomes caused temporal decrease in body weight; however, the body weight of mice became similar in both WT and GPNMB-KO mice of vehicle- and clodronate-injection group after 12 weeks of HFD. Macrophage depletion by clodronate liposomes completely abolished the aggravated obesity-related metabolic disorders in GPNMB-KO mice. Histological analysis showed less crown-like structures and decrease in macrophage infiltration, especially for CD11c-positive recruited macrophages, in the WAT of clodronate liposome injected mice, and macrophage infiltration in the WAT became similar in WT and GPNMB-KO mice after clodronate injection. Consistently, inflammatory cytokines expressions were reduced, and their enhanced expressions in the WAT of GPNMB-KO mice were disappeared after clodronate injection. These data indicate that loss of GPNMB causes aggravated obesity-related metabolic disorders largely through dysregulated macrophages in the WAT.

The candidate, having completed studies on the contribution of GPNMB to the development of obesity-induced insulin resistance and having advanced the field of knowledge in the area of the obesity-induced insulin resistance, is hereby recognized as having qualified for the degree of Ph.D.(Medicine).