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Blocking CXCR7-mediated adipose tissue macrophages chemotaxis attenuates insulin resistance and inflammation in obesity

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ABSTRACT

Adipose tissue macrophages (ATMs) have been considered to have a pivotal role in the chronic inflammation development during obesity. Although chemokine-chemokine receptor interaction has been studied in ATMs infiltration, most chemokine receptors remain incompletely understood and little is known about their mechanism of actions that lead to ATMs chemotaxis and pathogenesis of insulin resistance during obesity.

In this study, we reported that CXCR7 expression is upregulated in adipose tissue, and specifically in ATMs during obesity. In addition, CXCL11 or CXCL12-induced ATMs chemotaxis is mediated by CXCR7 in obesity but not leanness, whereas CXCR3 and CXCR4 are not involved. Additional mechanism study shows that NF-κB activation is essential in ATMs chemotaxis, and manipulates chemotaxis of ATMs via CXCR7 expression regulation in obesity. Most importantly, CXCR7 neutralizing therapy dose dependently leads to less infiltration of macrophages into adipose tissue and thus reduces inflammation and improves insulin sensitivity in obesity. In conclusion, these findings demonstrated that blocking CXCR7-mediated ATMs chemotaxis ameliorates insulin resistance and inflammation in obesity.

1. Introduction

Recent changes in human lifestyle have resulted in a marked increase in the incidence of obesity. Obesity, insulin resistance, and type 2 diabetes, continue to increase in incidence and are major contributors to morbidity and mortality worldwide [1]. There is accumulating evidence that expansion of adipose tissue mass is closely associated with low-grade chronic inflammation, to which has been given rise by the recruitment of kinds of immune cells [2]. In particular, adipose tissue macrophages (ATMs) have been considered to have a pivotal role in the chronic inflammation development during obesity. More specifically, ATMs as a percentage of total cells in the adipose tissue, expand from ~10% in lean individuals to more than 50% in those with advanced obesity, secrete proinflammatory cytokines such as TNF-α and CCL2, contributing to both local and systemic inflammation, thus potentiating insulin resistance and type 2 diabetes [3].

A number of hypotheses regarding the triggers of ATMs accumulation have been by far the most investigated in obesity. In this regard, several groups have studied the role of chemokine-chemokine receptor interaction including CCL2-CCR2, CCL5-CCR5, and CXCL5-CXCR2 [4–6], which have been involved in ATMs infiltration and pathogenesis of insulin resistance. Again, recent studies indicate that additional chemokines and their receptors may also be of great importance [7]. The complexity and redundancy of the chemokine system may account for the delicate regulation in ATMs infiltration and pathogenesis of insulin resistance. However, most chemokine receptors remain incompletely understood and little is known about their mechanism of actions that lead to ATMs infiltration [8].

One such chemokine receptor could be CXCR7. Very recently, CXCR7 have been found to express in adipose tissue, and its ligands CXCL11 and CXCL12 upregulated under condition of chronic inflammation during obesity [7]. In addition, many papers demonstrated that CXCR7 is increased upon macrophages activation and mediates signaling after its ligands CXCL11 and CXCL12 stimulation [9], thereby regulating macrophage chemotaxis. These intriguing findings propose a picture that CXCR7 might add another layer of its critical role of macrophage regulation of chronic inflammation in obesity. Although conflicting results raise debate whether CXCR7 acts as a signaling or non-signaling "decoy"

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receptor [10], accumulating results suggest that CXCR7 is not only physically interact with CXCL11 and CXCL12, but also be capable of turning on as a signal to trigger chemotaxis of macrophages, cancer cell and neuron migration [9,11].

Intrigued by these findings, we were interested to explore the important roles of CXCR7 and its implications in the inflammation and insulin resistance in obesity.

2. Materials and methods

2.1. Animals

Six-week-old male C57BL/6J mice were purchased from Vital River. Mice received either normal chow diet (NCD) or high fat diet (HFD) (Research Diets, 60 kcal% fat for the HFD), from 8 weeks of age on. All mice were housed in a specific pathogen-free facility with a 12-h light/dark cycle at our campus. All experimental procedures and protocols were approved by the Institutional Authority for Laboratory Animal Care of Shangqiu Medical College.

For CXCR7 neutralizing treatment, mice were divided randomly into four groups (n = 8 in each group), HFD-fed 8 weeks with (a) sheep IgG isotype control (R&D Systems) (10 μg); (b) anti-CXCR7 antibody (R&D Systems) (0.1 μg); (c) anti-CXCR7 antibody (1 μg); (d) anti-CXCR7 antibody (10 μg). Treatments were given by intraperitoneal injection twice a week from 8 to 16 weeks of age in mice with diet-induced obesity. At the end of the study, mice were sacrificed. ATMs isolated in the equal amount of adipose tissue were then counted by chemiluminescence using the luciferase-containing reagent Cell-Titer Glo (Promega).

2.2. Cell preparation, culture and stimulation

Fat pads of mice were excised and minced in PBS with calcium chloride and 0.5% BSA. Tissue suspensions were centrifuged at 500*g* for 5 min to remove erythrocytes and free leukocytes. Collagenase (Sigma-Aldrich) was added to 1 mg/mL and incubated at 37 C for 20 min with shaking. The cell suspension was filtered through a 100-μm filter and then spun at 300 g for 5 min to separate floating adipocytes from the SVF pellet. To ensure proper isolation, adipocyte fractions were examined by microscopy before and after plating on plastic to detect adherent cells. Samples were digested until adipocyte fractions were free of adherent cells to ensure recovery of the majority of the SVF population.

ATMs were identified by coexpression of F4/80 and CD11b in SVF isolated from NCD- or HFD mice for 16 weeks, and the cells were purified by positive selection on magnetic columns to select for $CD11b^{+}F4/80^{+}$ cells (Miltenyi Biotec) following the manufacturer's instructions. In some experiments, the cells in the flow through part were also pooled to collect the SVF cells without $CD11b^{+}F4/80^{+}$ cells. Cultures were maintained in an incubator at 37 °C in an atmosphere of 5% CO_2 and 95% air.

2.3. Real-time reverse transcription PCR (RT-PCR)

Total RNA was isolated using Trizol reagent according to the manufacturer's protocol, and 2 μg of total RNA was converted to cDNA by SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen). Brilliant SYBR-Green was combined with cDNA and corresponding primers (Supplementary Table. S1) specific for indicated genes and real-time PCR amplification was performed using the ABI Prism 7000 (Applied Biosystems). The expression levels of target genes were measured using the comparative Ct method (ddCt) normalized against β-actin.

2.4. Flow cytometry

Details are given in the Supplementary material and methods.

2.5. Enzyme-linked immunosorbent assay (ELISA)

2.5.1. ELISA for TNF-α and MCP-1

TNF-α and MCP-1 were measured in the supernatants using ELISA according to the manufacturer's instruction (eBioscience).

2.5.2. ELISA for total and phosphorylated AKT

Total and phosphorylated AKT was measured using AKT (Total/Phospho (Ser 473)) InstantOne™ ELISA (eBioscience) following the manufacturer's instructions. Briefly, cells were lysed in 1 × Cell Lysis Mix by incubation for 10 min at room temperature with shaking (300 rpm). Total soluble protein was quantified by BCA Protein Assay Kit and the same amount of protein was loaded per well (1 μg/well). 50 μL of antibody cocktail (capture antibody + detection antibody reagents from the ELISA kit) were then added and incubated for 1 h at room temperature. After three washes with 200 μL 1 × Wash Buffer per well, 100 μL of Detection Reagent were added and incubated for 30 min at room temperature. Finally, the reaction was terminated by adding 100 μL of Stop Solution and the plate was read by measuring absorbance at 450 nm.

2.5.3. ELISA for active NF-κB

The amount of activated NF-κB in nuclear extract (5 μg), prepared from 5×10^5 cells was assessed by a sensitive ELISA assay for active NF-κB (TRANS-AM, Active Motif). The NF-κB ELISA kits contain a 96-well plate on which has been immobilized with oligonucleotide containing the NF-κB consensus site (5′-GGGACTTTCC-3′). The active form of NF-κB contained in nuclear-cell extract specifically binds to this oligonucleotide. The primary antibodies used to detect NF-κB recognize an epitope on NF-κB that is accessible only when NF-κB is activated and bound to its target DNA. A horseradish peroxidase (HRP)-conjugated secondary antibody provides a sensitive colorimetric readout that is quantified by spectrophotometry b**y** reading within 5 min the absorbance at 450 nm.

2.6. Chemotaxis assay

Chemotaxis assays were performed using 6.5-mm Transwell tissue culture inserts with a 5 μm pore size (Corning). ATMs were suspended at 1×10^6 cells/ml in DMEM with 0.1% BSA, and 100 μL of cell suspension was added to an insert in a well. The lower compartment was placed with 600 μL of medium containing CXCL11 or CXCL12, and the plates were then incubated for 180 min. Macrophages found on the bottom of filters were counted as cells that had carried out chemotaxis. Cells were fixed with 100% methanol, and stained with Giemsa. The migrant cells were counted in five randomly selected high-power fields (400 ×) per well. The chemotaxis index was calculated as the number of cells that migrated to the sample medium divided by the number of cells that migrated to the control medium. For the chemotaxis inhibition assay, cells were pretreated with indicated inhibitors, a neutralizing anti-CXCR7 antibody or sheep IgG isotype control, and then loaded into the upper chamber. To specifically inhibit activity of CXCL11 or CXCL12, we added the corresponding neutralizing antibody (anti-SDF-1 antibody, clone#79014; anti-I-TAC antibody, clone#131327, R&D Systems) to the adipocyte supernatants.