Blocking gp130 signaling suppresses autotaxin expression in adipocytes and improves insulin sensitivity in diet-induced obesity

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Abstract Autotaxin (ATX), which is highly expressed and secreted by adipocytes, functions as the key enzyme to generate lysophosphatidic acid (LPA) from lysophosphatidylcholine. Adipose tissue is the main source of circulating ATX that modulates plasma LPA levels. Upregulation of ATX expression in obese patients and mice is closely related with insulin resistance and impaired glucose tolerance. However, the mechanism of ATX expression in adipocytes remains largely unknown. In this study, we found that glycoprotein 130 (gp130)-mediated Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3) activation was required for abundant ATX expression in adipocytes. Through gp130, the interleukin 6 (IL-6) family cytokines, such as IL-6, leukemia inhibitory factor, cardiotrophin-1, and ciliary neurotrophic factor, upregulated ATX expression in adipocytes. ATX contributes to the induction of insulin resistance and lipolysis in IL-6-stimulated adipocytes. Oral administration of gp130 inhibitor SC144 suppressed ATX expression in adipose tissue, decreased plasma ATX, LPA, and FFA levels, and significantly improved insulin sensitivity and glucose tolerance in high-fat diet-fed obese mice. In summary, our results indicate that the activation of gp130-JAK-STAT3 pathway by IL-6 family cytokines has an important role in regulating ATX expression in adipocytes and that gp130 is a promising target in the management of obesity-associated metabolic diseases.—Sun, S., R. Wang, J. Song, M. Guan, N. Li, X. Zhang, Z. Zhao, and J. Zhang. Blocking gp130 signaling suppresses autotaxin expression in adipocytes and improves insulin sensitivity in diet-induced obesity. J. Lipid Res. 2017. 58: 2102–2113.

Supplementary key words glycoprotein 130 • autotaxin • adipocyte • insulin sensitivity • obesity

Autotaxin (ATX) is a secreted glycoprotein, originally identified as an autocrine motility-stimulating factor produced by cancer cells (1). ATX catalyzes the conversion of lysophosphatidylcholine to lysophosphatidic acid (LPA) (2), which is a bioactive lysophospholipid acting via at least six G protein-coupled receptors, termed LPA receptors 1–6 (LPA1–LPA6), on cell membranes (3). The ATX-LPA axis participates in the regulation of various cellular activities such as cell proliferation, migration, vascular development, and tissue differentiation (4, 5). In ATX−/− mice, knockout is lethal because of vascular and neural tube defects in embryogenesis (6–8), while ATX+−/− mice are apparently healthy with plasma LPA levels approximately half of those found in WT mice (9), indicating that ATX functions as a key enzyme in LPA generation.

ATX is abundantly expressed and secreted by adipocytes. Adipocyte-specific ATX knockout mice have shown a 38% reduction in plasma LPA levels compared with WT mice (10), a finding that suggests that adipose is the major source of circulating ATX for the regulation of plasma LPA levels. ATX expression in adipose tissue and plasma LPA levels are upregulated in high-fat diet (HFD)-fed obese mice (11). Adipocyte-specific ATX knockout mice fed a HFD display less insulin resistance than WT mice fed the same diet (12), suggesting that ATX from adipose tissue contributes to the impaired glucose homeostasis observed in diet-induced obesity. However, the mechanism that underlies the regulation of ATX expression in adipocytes is largely unknown.

Abbreviations: ATX, autotaxin; CNFF, ciliary neurotrophic factor; CT-1, cardiotrophin-1; gp130, glycoprotein 130; GTT, glucose tolerance test; HFD, high-fat diet; HSL, hormone-sensitive lipase; IL-6, interleukin 6; IL-11, interleukin 11; IP, intraperitoneal; ITT, insulin tolerance test; JAK, Janus kinase; LIF, leukemia inhibitory factor; LPA, lysophosphatidic acid; LPA1-6, lysophosphatidic acid receptors 1-6; ND, normal diet; P-STAT3, phosphorylated signal transducer and activator of transcription 3; RT-qPCR, real-time quantitative PCR; STAT, signal transducer and activator of transcription.

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The interleukin 6 (IL-6) cytokine family is a group of functional secreted proteins that include IL-6, interleukin 11 (IL-11), leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), and neuropoietin. Glycoprotein 130 (gp130) is the common subunit of the receptor signaling complex for the IL-6 family cytokines (13–15). Ligand binding induces association of gp130 with the cytokine-specific receptor, followed by activation of the downstream signaling pathway. Each cytokine has a specific receptor. For instance, IL-6 and IL-11 initially bind to IL-6 receptor 

Before forming a complex with a gp130 homodimer for signaling, whereas LIF and CT-1 require a complex consisting of gp130 and the LIF receptor to mediate signal transduction (16, 17). The IL-6 cytokine family is involved in signaling via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and other signaling pathways such as RAS/MAPK and PI3K/Akt (18, 19). gp130 is ubiquitously expressed, and activation of gp130 signaling by the IL-6 family cytokines affects the regulation of a wide range of important biological processes, including inflammation, autoimmunity, cancer, and embryonic development (20, 21).

In recent years, studies have shown that adipose tissue is not only an energy storage tissue, but also an osteochondral organ producing numerous adipokines. IL-6 is an adipokine, because approximately one-third of total IL-6 expression in healthy humans is estimated to be derived from adipose tissue (22, 23). Circulating IL-6 levels are greatly elevated in obese humans and are correlated with insulin resistance (24, 25). The biological activities of adipocytes are regulated by IL-6 through the IL-6 receptor and gp130 receptor. IL-6 treatment can induce insulin resistance and promote lipolysis in adipocytes (26, 27). In this study, we demonstrate that activation of the gp130-JAK-STAT3 pathway by IL-6 and the other members of the IL-6 cytokine family has an important role in the upregulation of ATX expression in adipocytes. Meanwhile, ATX contributes to the induction of insulin resistance and lipolysis in IL-6-stimulated adipocytes. Oral administration of the gp130 inhibitor, SC144, blocked the increase of ATX expression in adipose tissue, suppressed elevation of plasma ATX, LPA, and FFA levels, and improved both insulin sensitivity and glucose tolerance in HFD-fed obese mice. These findings indicate that gp130 is a promising target in the management of obesity-related glucose metabolic diseases.

MATERIALS AND METHODS

Cell culture

The 3T3-L1 preadipocytes were maintained in DMEM (Macne, China) supplemented with 10% newborn calf serum (NCS; Gibco), 100 U/ml penicillin, and 100 U/ml streptomycin (Life Technologies). Adipocyte differentiation was performed as described previously (28). Briefly, 3T3-L1 preadipocytes were incubated in DMEM containing 10% FBS (Gibco), 0.5 mM 3-Isobutyl-1-methyl-xanthin (IBMX; Sigma-Aldrich), 0.25 μM dexamethasone (DEX; Sigma-Aldrich), and 5 μg/ml insulin (Sigma) for 2 days, followed by incubation in DMEM containing 10% FBS and 5 μg/ml insulin with fresh medium replaced every 2 days until cells exhibited an adipocyte morphology. Mature 3T3-L1 adipocytes were maintained in DMEM with 10% FBS. For experiments that measured secreted ATX, cells were cultured in serum-free medium for 24 h. All cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂, MDA-MB-435 (melanoma), U87 (glioblastoma), HeLa (cervical carcinoma), MCF-7 (breast cancer), and HEK293 cell lines were maintained in DMEM supplemented with 10% FBS, and the HT29 colorectal cancer cell line was maintained in McCoy’s 5a (Macne, China) supplemented with 10% FBS.

Reagents and antibodies

Insulin, DEX, IBMX, and the JAK inhibitor AG490 were purchased from Sigma-Aldrich. The gp130 inhibitor SC144, STAT3 inhibitor S3I-201, and LPA1/3 antagonist Ki16425 were purchased from Selleckchem. Recombinant mouse IL-6, IL-11, LIF, CT-1, and CNTF were purchased from PeproTech. The primary antibody for ATX was generated by our lab as described previously (29). Antibodies to STAT3 (sc-482) and PPAR-γ (sc-166731) were purchased from Santa Cruz Biotechnology (Santa Cruz). Antibodies to STAT1 (14994), phosphorylated STAT1 (P-STAT1) (9167), P-STAT3 (9145), and gp130 (3792) were purchased from Cell Signaling Technology. The antibody to GAPDH (CW0100M) was obtained from Cwbio (Beijing, China).

siRNA transfection

All siRNAs were synthesized by GenePharma (Shanghai, China). The target sequences were listed in supplemental Table S1. siRNAs were transfected into 3T3L1 adipocytes with Lipofectamine 2000 (Invitrogen), according to the protocol supplied by the manufacturer. The siRNA concentration for transfection was about 100 nM. In each siRNA transfection experiment, nonspecific siRNA (NC) was used as a normal control.

Western blot analyses

Cells were lysed in RIPA buffer for 30–60 min. After centrifugation (13,500 g, 30 min) at 4°C, the supernatant was collected and washed with PBS three times, and then homogenized and lysed in RIPA buffer. After centrifugation (13,500 g, 30 min) at 4°C, the supernatant was collected as tissue lysate. Cell lysates and tissue lysates were quantified by the BCA assay (Micro BCA; Pierce Biotechnology). To detect ATX in culture medium, identical volumes of serum-free conditioned culture medium were concentrated (approximately 30-fold) by using an Amicon Ultra 30000 (Millipore). Protein samples were separated by using SDS-PAGE and analyzed by Western blot as described previously (30).

RNA extraction and quantitative RT-PCR

Total RNA was extracted from cells by using Trizol reagent (Sigma). RNA (2 μg) isolated from cells was reverse-transcribed by using anchored oligo dT primers and the Reverse Transcription System (Promega). cDNAs encoding the indicated genes were amplified with specific primers. The primes are listed in supplemental Table S2. Each real-time quantitative PCR (RT-qPCR) experiment was repeated at least three times using samples in triplicate. RT-qPCR was performed by using SYBR Green Supermix (Thermo Fisher Scientific) with the iCycler IQ5 Real-Time PCR Detection System (Bio-Rad). Relative expression of each target gene was estimated by normalization with the expression level of GAPDH.
Lipid extraction and analysis
Lipids were extracted by the methanol method (31). In brief, 20 μl of plasma was transferred into 1,000 μl of methanol containing 14:0 LPA (20 pmol, used as internal standard). After vortexing and centrifugation (10,000 g, 5 min, room temperature), 2 μl of the supernatant was loaded into a mass spectrometer for lipid analysis. Lipids analysis was performed by using the API 4500 QTRAP mass spectrometer (Applied Biosystems/MDS SCIEX) combined with a liquid chromatography system (1-class Acquity ultra performance liquid chromatography, Waters). Standard curves were established for quantitative analyses of all lipids (32).

Determination of plasma FFA levels
The extraction of FAs from plasma was prepared as in the method reported previously (31). In brief, 20 μl of plasma was mixed with 2 μl of 17:0 FA (internal standard, 500 μM), then added into 480 μl of methanol. The mixture was vortexed for 1 min and then centrifuged by 3,000 g for 10 min. The supernatant was collected and dried under nitrogen at room temperature. The dryness was derivatized and then analyzed by MS according to the work of Johnson and Pettinella et al. (33, 34). Ultra-performance liquid chromatography (1-class Acquity ultra performance liquid chromatography, Waters) with mass spectrometry (AB SCIEX QTrap 4500) was used for quantitation compared to the peak area of FAs with that of internal standard (17:0 FA).

Animal experiments
All mice had a C57BL/6 background and were housed under a 12 h light-dark cycle with ad libitum access to food and water. Six-week-old C57BL/6 mice were divided into four groups (five mice per group). Mice in group 1 were fed a normal diet (ND), termed the ND group; group 2 was fed a HFD (60 kcal% fat, Research Diets) for 8 weeks, known as the HFD group; group 3 was fed a HFD for 8 weeks with daily oral administration of the gp130 inhibitor SC144 (10 mg/kg, dissolved with 0.9% NaCl with 40% propylene glycol) in the eighth week, termed the HFD+SC144 group; and group 4 was fed a HFD for 8 weeks with daily intraperitoneal (IP) injection of the LPA1/3 antagonist Ki16425 (5 mg/kg, dissolved with 0.9% NaCl with 40% propylene glycol) in the eighth week, known as the HFD+Ki16425 group. Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were performed at the end of the eighth week to assess glucose and insulin tolerance. For GTT, mice were subjected to IP injection of glucose (1 g/kg, after 12 h fasting), and blood glucose concentrations (milligrams per desiliter) were measured every 15 or 30 min as indicated. For ITT, mice were subjected to IP injection of insulin (0.75 U/kg, after 12 h fasting), and blood glucose concentrations were measured every 15 or 30 min as indicated. Mice were sacrificed to isolate tissues at the end of the eighth week. RNA extracted from adipose tissue was used to measure ATX mRNA expression level by RT-qPCR, and LC MS/MS was performed to measure plasma ATX, LPA, and FFA levels. All animal experiments were performed using a protocol approved by the Animal Studies Committee at the Beijing Normal University in Beijing, China.

Statistics
Data are presented as means ± SEM. Student’s t-tests were used to compare two groups of data. Differences among multiple groups were evaluated using ANOVA followed by Bonferroni post hoc tests. Values of P < 0.05 were considered significant.

RESULTS
Activation of the gp130-JAK-STAT3 signaling pathway is required for high ATX expression in adipocytes
The 3T3-L1 preadipocytes were induced to differentiate into adipocytes in vitro by using PPARγ as a positive marker of adipocyte differentiation. Consistent with a previous study (35), it was found that ATX expression and secretion were significantly increased during adipogenesis and were maintained at a high level in mature adipocytes (Fig. 1A and supplemental Fig. S1). To explore the mechanism of ATX expression in adipocytes, ATX expression levels were measured after treatment with several signal pathway inhibitors. It was found that ATX expression in adipocytes was significantly inhibited by SC144, a specific inhibitor of gp130, in a time- and dose-dependent manner (Fig. 1B and supplemental Fig. S2A, B). In addition, downregulation of gp130 by siRNA suppressed ATX expression in adipocytes (Fig. 1C).

gp130 is the common subunit of the receptor signaling complexes of the IL-6 cytokine family, which includes IL-6, IL-11, IL-27, LIF, CNTF, OSM, and CT-1. These cytokines share gp130 to activate different downstream signaling pathways such as JAK-STAT and MAPK (18). The JAK inhibitor, AG490, blocked ATX expression in adipocytes in a time- and dose-dependent manner (Fig. 2A and supplemental Fig. S2C, D), but inhibition of ERK1/2, JNK, or p38 MAPK activation by their specific inhibitor (PD169316 10 μM, SP600125 20 μM, and SB239063 5 μM) had no effect on ATX expression in adipocytes (data not shown). The mammalian JAK protein family has four members, JAK1, JAK2, JAK3, and TYK2. Knockdown of JAK1, JAK2, or TYK2, but not JAK3, resulted in downregulation of ATX expression in adipocytes (Fig. 2B). Furthermore, coknockdown of JAK1, JAK2, JAK3, and TYK2 significantly inhibited ATX expression in adipocytes (Fig. 2C). These results suggest that JAK1/2 and TYK2 are involved in ATX expression regulation in adipocytes. JAK activates STAT family members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6), among which STAT1 and STAT3 have important roles in adipocyte differentiation and adipocyte functions (16, 36). ATX expression in adipocytes was downregulated by STAT3 knockdown, but not by STAT1 knockdown (Fig. 2D). In addition, the STAT3-specific inhibitor, S3I-201, did significantly decrease ATX expression in adipocytes (Fig. 2E). Compared with in preadipocytes, the level of P-STAT3, which is the active form of STAT3, was markedly higher in differentiated adipocytes (Fig. 1A). Inhibition of gp130 and JAK activation by the corresponding inhibitor or siRNA led to downregulation of P-STAT3 levels in adipocytes (Figs. 1B, C and 2A, C). Collectively, these findings demonstrate that activation of the gp130-JAK-STAT3 pathway is required for the high level of ATX expression found in adipocytes.

ATX is upregulated by the IL-6 family cytokines and involved in the induction of insulin resistance and lipolysis in adipocytes
IL-6 family cytokines are referred to as gp130 cytokines because all members of this family utilize the gp130...
subunit as a common signal transducer within their respective receptor complex. Because the gp130-JAK-STAT3 signal pathway is involved in regulating ATX expression in adipocytes, the role of each IL-6 family cytokine on ATX expression was further characterized. We found that several members of the IL-6 cytokine family, including IL-6, IL-11, LIF, CT-1, and CNTF, are expressed in 3T3L1 adipocytes (supplemental Fig. S3). Following treatment of 3T3-L1 adipocytes with different IL-6 cytokines, it was found that IL-6, LIF, CT-1, and CNTF increased P-STAT3 levels and upregulated ATX expression (Fig. 3A–D). However, IL-11 treatment increased P-STAT1, but not P-STAT3, levels, and did not elevate ATX expression (Fig. 3E). Upregulation of ATX expression by IL-6 was blocked by gp130, JAK, or STAT3 inhibitor, which also resulted in a decreased P-STAT3 level (Fig. 3F). Based on these findings, we propose that the IL-6 family cytokines are expressed and secreted by adipocytes and function as autocrine factors to maintain a high level of ATX expression.

Adipose tissue is a major site of IL-6 production. The IL-6 receptor and gp130 are expressed in adipocytes to mediate the action of IL-6 on fat cells. It has been reported that IL-6 treatment can induce insulin resistance in adipocytes (26). In the presence of IL-6, the insulin-stimulated Akt phosphorylation was suppressed in 3T3L1 adipocytes. The suppression of insulin-induced Akt activation by IL-6 was relieved by the treatment with ATX inhibitor S32826 (Fig. 3G). IL-6 also functions as a potent stimulator to promote lipolysis in adipocytes (27). Here, we found that the levels of phosphorylated hormone-sensitive lipase (HSL) in adipocytes were increased by IL-6 treatment, while the activation of HSL by IL-6 was inhibited in the presence of ATX inhibitor S32826 (Fig. 3H). In addition, LPA treatment could suppress the insulin-induced Akt phosphorylation and promote the activation of HSL in adipocytes (supplemental Fig. S4).

These data suggest that the upregulation of ATX expression contributes to the development of insulin resistance and the promotion of lipolysis in IL-6-stimulated adipocytes.

Obesity is a chronic inflammatory process, during which immune cells such as myeloid and lymphoid cells infiltrate adipose tissue. The expression of inflammatory cytokines, such as IL-6 and TNF-α, in adipose tissue is increased in obesity and is associated with inflammation in adipose tissue (37, 38). Interestingly, when 3T3-L1 adipocytes were treated with IL-6 and TNF-α together, ATX expression was upregulated to a significantly higher level compared with that found using IL-6 or TNF-α alone (Fig. 3I). Activation of NFκB in adipocytes by TNF-α may contribute to upregulation of...
ATX, because ATX expression in adipocytes is downregulated by the NFκB inhibitor PDTC (20 μM) (supplemental Fig. S5). These findings indicate that the IL-6 cytokine family can cooperate with other cytokines to regulate ATX expression in adipocytes.

gp130 is universally expressed in various types of cells. To test cell type-specific regulation of ATX by the IL-6 family cytokines, cells from the cell lines MDA-MB-435, U87, HEK293, HeLa, HT29, and MCF-7 were treated with IL-6. The endogenous ATX expression is relatively high in MDA-MB-435 and U87 cells, and very low in HEK293, HeLa, HT29, and MCF-7 cells (supplemental Fig. S6). It was found that IL-6 upregulated ATX expression in MDA-MB-435 and U87 cells. However, in HEK293, HeLa, HT29, and MCF-7 cells, IL-6 treatment increased P-STAT3 levels but did not upregulate ATX expression (Fig. 3J). Our data indicate that the regulation of ATX expression by the IL-6 family cytokines is cell type-dependent.

Oral administration of gp130 inhibitor SCI144 inhibits ATX expression in adipose tissue and decreases plasma ATX and LPA levels in HFD-fed obese mice

Male mice were fed a HFD or ND for 8 weeks. During the eighth week, the gp130 inhibitor SCI144 (5 mg/kg) was orally delivered daily to one group of HFD-fed mice (HFD+SCI144), with another group of HFD-fed mice as the untreated control. HFD feeding increased body and fat pad weights, whereas treatment with gp130 inhibitor SCI144 did not affect the increase of body weight and fat pad weight (Fig. 4A). The ATX mRNA levels in epididymal adipose tissue were detected by real-time RT-qPCR. Compared with ND mice, HFD mice had significantly higher ATX expression level in adipose tissues. Oral administration of SCI144 resulted in a decrease of ATX mRNA levels in the adipose tissues of HFD obese mice, down to about the levels in the adipose tissue of ND mice (Fig. 4B). After detection of STAT3/P-STAT3 levels in epididymal adipose tissue, it was found that P-STAT3 levels in adipose tissue of HFD mice were considerably higher than those found in ND mice. P-STAT3 levels in adipose tissue of HFD mice were decreased after SCI144 treatment, without significant changes in the total level of STAT3 (Fig. 4C). It has been demonstrated that adipose tissue is the main source of plasma ATX (10, 12). In fact, we found that plasma ATX levels in HFD mice were significantly decreased by SCI144 treatment (Fig. 4C). In addition, HFD mice had higher plasma LPA and S1P concentrations than those found in ND mice (Fig. 4D, E). Plasma levels of all major LPA species in HFD mice were downregulated by SCI144 treatment (supplemental Fig. S7), which resulted in the significant decrease of plasma LPA level observed in HFD mice (Fig. 4D). Meanwhile, plasma levels of another important bioactive lipid, S1P, had no changes among the HFD and HFD+SCI144 groups (Fig. 4E). Similar experimental findings...
Fig. 3. Role of the IL-6 family cytokines in regulating ATX expression. A–D: Effects of administration of different members of the IL-6 family cytokines on ATX expression in adipocytes. The 3T3-L1 adipocytes were starved overnight followed by incubation with fresh medium containing the indicated IL-6 family cytokine. The 3T3-L1 adipocytes were treated with mouse recombinant IL-6 (10 ng/ml), LIF (10 ng/ml), CT-1 (10 ng/ml), and CNTF (10 ng/ml) for 8 h. RNA isolated from cells was subjected to RT-qPCR to assess ATX mRNA levels. ATX protein levels in culture medium (CM) and STAT3/P-STAT3 protein levels in cell lysates were detected by Western blot. E: Treated 3T3-L1 adipocytes with IL-11 (10 ng/ml) for 8 h. RNA isolated from cells was subjected to RT-qPCR to assess ATX mRNA levels. ATX protein levels in culture medium (CM) and STAT3/P-STAT3 and STAT1/P-STAT1 protein levels in cell lysates were detected by Western blot. F: Upregulation of ATX expression in adipocytes by IL-6 was blocked by gp130, JAK, and STAT3 inhibitors. Starved 3T3-L1 adipocytes were pretreated with the gp130 inhibitor SC144 (10 μM), the JAK inhibitor AG490 (50 μM), or the STAT3 inhibitor SI-301 (20 μM) for 30 min, followed by treatment with IL-6 (10 ng/ml) for 8 h. ATX mRNA levels were assessed by RT-qPCR, and ATX protein levels in culture medium (CM) and STAT3/P-STAT3 protein levels in cell lysates were detected by Western blot. G: 3T3-L1 adipocytes were starved overnight, followed by IL-6 (20 ng/ml) treatment with or without ATX inhibitor S32826 (2 μM) for 24 h and then incubated with insulin (200 nM) for 15 min. AKT and P-AKT in cell lysates were detected by Western blot. H: The 3T3-L1 adipocytes were starved overnight, followed by IL-6 (20 ng/ml) treatment with or without ATX inhibitor S32826 (2 μM) for 24 h. HSL and P-HSL in cell lysates were detected by Western blot. I: Synergistic effect of TNF-α on ATX upregulation in adipocytes. The 3T3-L1 adipocytes were treated for 8 h with IL-6 (10 ng/ml) and/or TNF-α (50 ng/ml) as indicated. ATX mRNA levels were assessed by RT-qPCR. ATX protein levels in culture medium (CM) and STAT3/P-STAT3 and P65/P-P65 protein levels in cell lysates were detected by Western blot. J: Cell type-specific regulation of ATX expression by IL-6. Cells from the human melanoma cell line MDA-MB-435, the human malignant glioma cell line U87, the human kidney epithelial cell line HEK 293, the human cervical cancer cell line HeLa, the human colon cancer cell line HT-29, and the human breast cancer cell line MCF-7 were treated with or without IL-6 (10 ng/ml) for 8 h. ATX mRNA levels were assessed by RT-qPCR, and ATX protein levels in culture medium (CM) and STAT3/P-STAT3 protein levels in cell lysates were detected by Western blot. Data are representative of three independent experiments. Values of \( P < 0.05 \) were considered statistically significant. * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.005 \); N.S., not significant (Student's t-test in A–E and J; ANOVA in F and I).
Fig. 4. Effects of SC144 oral administration on ATX expression in adipose tissue and plasma ATX and LPA levels in HFD-fed obese male mice. C57BL/6 male mice were fed a ND or HFD for 8 weeks. During the eighth week, the gp130 inhibitor SC144 (5 mg/kg) was orally delivered daily to one group of HFD-fed mice (HFD+SC144), with another group of HFD-fed mice as the untreated control. A: Mouse body weights were measured weekly, and fat pad weights of epididymal depots were measured at the end of the eighth week. B: ATX mRNA levels in adipose tissue of ND, HFD, and HFD+SC144 mice. At the end of the eighth week, RNA isolated from epididymal adipose tissue was subjected to RT-qPCR to assess ATX mRNA levels. C: STAT3/P-STAT3 levels in adipose tissue and ATX protein levels in plasma of ND, HFD, and HFD+SC144 mice. At the end of the eighth week, STAT3/P-STAT3 levels in epididymal adipose tissue and ATX protein levels in plasma were detected by Western blot. P-STAT3 protein was normalized to total STAT3 protein and presented as fold of the ND baselines. D–E: Plasma LPA and S1P levels of ND, HFD, and HFD+SC144 mice. At the end of the eighth week, plasma samples were collected and plasma concentrations of LPA (D) and S1P (E) were measured by LC-MS/MS analysis as described in the Materials and Methods. F: Expression levels of the IL-6 family cytokines in adipose tissue of ND, HFD, and HFD+SC144 mice. At the end of the eighth week, RNA isolated from epididymal adipose tissue was subjected to RT-qPCR to assess IL-6, LIF, CT-1, and CNTF mRNA levels. *HFD group versus ND group; #HFD group versus HFD+SC144 group; §HFD+SC144 group versus ND group. n = 5 animals in each group. The statistical significance of differences among groups was evaluated using ANOVA test. Values of $P < 0.05$ were considered statistically significant. *,#,$ P < 0.05$, and **,##,$§ P < 0.01$. 
HFD mice had higher levels of IL-6 and LIF expression in adipose tissue compared with those found in ND mice. IL-6 and LIF mRNA levels in adipose tissue and IL-6 levels in plasma of HFD mice were not significantly affected by SC144 treatment (Fig. 4F and supplemental Fig. 59). The expression levels of two other members of the IL-6 family of cytokines, CT-1 and CNTF, had no significant differences between mice from the ND, HFD, and HFD+SC144 groups (Fig. 4F). These results suggest that IL-6 and LIF may be the major gp130 cytokines for upregulation of adipose ATX expression and elevation of plasma ATX and LPA levels in HFD-induced obesity.

SC144 treatment and Ki16425 treatment inhibit macrophage infiltration of adipose tissue, decrease plasma FFA levels, and improve insulin sensitivity and glucose tolerance in HFD-fed obese mice

Obesity is often associated with a prediabetic state characterized by alterations in glucose homeostasis, including impaired glucose tolerance and insulin resistance (39). Deletion of ATX in adipocytes reduces plasma LPA and improves both insulin sensitivity and glucose tolerance in HFD-fed mice (40), findings that suggest ATX from adipose tissue contributes to the impaired glucose homeostasis found in diet-induced obesity. In this study, we have demonstrated that ATX expression in adipocytes is upregulated by IL-6 family cytokines through activating the gp130-JAK-STAT3 pathway. IL-6 is mainly produced from adipose tissue and involved in adipose tissue inflammation, fat lipolysis, and insulin resistance. In a further study, the effects of the gp130 inhibitor SC144 and LPA1/3 antagonist Ki16425 on adipose tissue macrophage infiltration, plasma FAA levels, and glucose homeostasis of HFD-fed obese mice were examined. Groups of mice were fed a ND or HFD for 8 weeks. In the eighth week, the HFD-fed obese mice were treated with SC144 (5 mg/kg, orally delivered daily for 1 week, termed HFD+SC144) or Ki16425 (10 mg/kg, daily for 1 week, termed HFD+Ki16425), with the untreated HFD mice as control. The ND, HFD control, HFD+SC144, and HFD+Ki16425 groups underwent tests at the end of the eighth week. As expected, the plasma FAA levels and the transcript levels of the macrophage markers CD11b and F4/80 in epididymal adipose tissue were increased in HFD-fed obese mice. These obesity-associated increases were reversed by the treatment with either SC144 or Ki16425 treatment (Fig. 5A–D). GTT and ITT results showed that the systemic insulin resistance and impaired glucose tolerance resulting from HFD-induced obesity were significantly ameliorated by the administration of either SC144 or Ki16425 (Fig. 5E, F). These findings indicate that blocking gp130 signaling has similar effects as inhibiting LPA1/3 signaling, to suppress adipose tissue inflammation, decrease plasma FAA levels, and improve glucose homeostasis in HFD-induced obese mice.

DISCUSSION

ATX is a secreted glycoprotein with lysophospholipase D activity and functions as the key enzyme in the generation of LPA, which is a bioactive lysophospholipid mediator, acting through its specific receptors on cell membranes to regulate a wide range of cellular activities, including cell proliferation, differentiation, apoptosis, and migration (4, 41). The ATX-LPA axis is involved in the regulation of various physiological and pathological processes, and, in particular, obesity, neural development, inflammation, and oncogenesis (5). ATX is abundantly expressed and secreted from adipocytes, and ATX from adipose tissues mainly contributes to plasma ATX and LPA levels (10, 35). However, the mechanism of high-level ATX expression in adipocytes remains poorly understood.

In the present study, we demonstrate that the gp130-JAK-STAT3 pathway has a key role in regulating ATX expression in adipocytes. Inhibition of gp130, JAK1, JAK2, TYK2, and STAT3 activation by their respective inhibitor, as well as their knockdown using a specific siRNA, reduced ATX expression in adipocytes. IL-6 family cytokines, also known as gp130-dependent cytokines, induce association of gp130 with the respective cytokine-specific receptor that results in the downstream activation of the JAK-STAT3 pathway. We found that the following members of the IL-6 cytokine family, IL-6, LIF, CT-1, and CNTF, were expressed by adipocytes and functioned as autocrine factors to induce the gp130-JAK-STAT3 pathway and upregulate ATX expression in adipocytes. However, one member of the IL-6 cytokine family, IL-11, which induces P-STAT1, but not P-STAT3, had no effect on ATX expression in adipocytes. It has been reported previously that STAT3 mediates ATX expression in several kinds of breast cancer cells (42), but ATX expression was not upregulated by STAT3 activation in the IL-6-treated MCF-7 cells. Therefore, although gp130 is universally expressed, our findings indicate that upregulation of ATX by IL-6 was cell type-dependent, the mechanism of which requires further study.

Adipose tissue in obesity is in a state of inflammation and is characterized by increased infiltration of macrophages, which are responsible for increased expression of proinflammatory cytokines (43–45). The inflammatory cytokines including TNF-α, granulocyte colony-stimulating factor, and IL-1β, have been reported to play a role in ATX expression regulation (46–48). In this study, we found that cotreatment of adipocytes with IL-6 and TNF-α resulted in upregulation of ATX that was markedly higher compared with that found using IL-6 or TNF-α alone, suggesting that IL-6 and TNF-α exert a synergistic effect on ATX expression in adipocytes. Therefore, inflammation in adipose tissue may contribute to the obesity-associated increase of ATX expression in adipocytes. It has been reported that adipose tissue inflammation is suppressed by adipocyte-specific...
Fig. 5. Effects of SC144 and Ki16425 treatment on plasma FFA levels, macrophage infiltration of adipose tissue, insulin sensitivity, and glucose tolerance in HFD-fed obese male mice. C57BL/6 male mice were fed a ND or a HFD for 8 weeks. During the eighth week, one group of the HFD-fed obese mice were treated with SC144 (5 mg/kg, orally delivered daily for 1 week, termed HFD+SC144) or Ki16425 (IP 5 mg/kg, daily for 1 week, termed HFD+Ki16425), with the untreated HFD mice as control. The ND, HFD control, HFD+SC144, and HFD+Ki16425 groups underwent tests at the end of the eighth week. A: Plasma samples of ND, HFD, and HFD+SC144 mice were collected at the end of the eighth week, and plasma concentrations of FFA were measured by LC MS/MS analysis. B: Plasma samples of ND, HFD, and HFD+Ki16425 mice were collected at the end of the eighth week, and plasma concentrations of FFA were measured by LC MS/MS analysis. C: F4/80 (left) and CD11b (right) mRNA levels in adipose tissue of ND, HFD, and HFD+SC144 mice. D: F4/80 (left) and CD11b (right) mRNA levels in adipose tissue of ND, HFD, and HFD+Ki16425 mice. At the end of the eighth week, epididymal adipose tissue was collected. RNA isolated from epididymal adipose tissue was subjected to RT-qPCR to assess F4/80 and CD11b mRNA levels. E: GTT (left) and ITT (right) of ND, HFD,
ATX deletion and by inhibition of ATX activity (12, 47). In this study, we found that macrophage infiltration of adipose tissue in HFD mice was decreased by treatment with either the gp130 inhibitor SC144 or the LPA1/3 antagonist Ki16425 (Fig. 5C, D). These results suggest that the gp130-mediated ATX expression in adipocytes is involved in the development of adipose tissue inflammation.

Both circulating ATX and IL-6 are mainly produced from adipose tissue. Correlated with the elevation of ATX expression, the expression of IL-6 family cytokines, IL-6 and LIF, are significantly increased in adipose tissue of HFD-fed obese mice (Fig. 4F). Although multiple cytokines participate in the regulation of ATX expression, we propose that the activation of gp130 signaling by IL-6 family cytokines, especially IL-6, would play an important role to promote ATX expression in adipocytes. IL-6 can induce insulin resistance and promote lipolysis in adipocytes. Here, we found that IL-6-induced insulin resistance and lipolysis in adipocytes were suppressed in the presence of ATX inhibitor (Fig. 3G, H), suggesting that the gp130-mediated upregulation of ATX in turn contributes to the induction of insulin resistance and lipolysis in IL-6-stimulated adipocytes.

When fed a HFD, mice with an adipocyte-specific ATX deletion showed improved glucose tolerance and insulin sensitivity compared with WT mice (12), indicating that adipose-derived ATX contributes to the obesity-related alteration found in glucose homeostasis. In this study, it was found that oral administration of gp130 inhibitor SC144 to HFD-fed obese mice suppressed ATX expression in adipose tissue and reduced ATX and LPA plasma concentrations to normal levels, confirming that gp130 signaling plays an important role in the regulation of ATX expression in adipocytes. Furthermore, blocking gp130 signaling by SC144 has similar effects as inhibiting LPA1/3 signaling by Ki16425, resulting in suppression of adipose tissue inflammation, decrease of plasma FFA levels, and improvement in glucose tolerance and insulin sensitivity in HFD-induced obese mice. It has been reported recently that gp130-mediated adipose tissue lipolysis promotes hepatic steatosis and insulin resistance (49). In this study, our data suggest that ATX-LPA signaling participates in the modulation of lipolysis. Meanwhile, there is evidence to support that ATX/LPA signaling has direct impact to impair glucose homeostasis (12, 40, 50). We propose that the inhibition of ATX expression and decrease of lipolysis both contribute to the improvement of insulin sensitivity and glucose tolerance in SC144-treated obese mice. Oral administration of SC144 has anticancer activity without significant toxicity to normal tissues (51). Our findings in this study reveal a novel activity of SC144 in the treatment of obesity-associated glucose metabolic disorders. Because the ATX-LPA axis has a role in oncogenesis by promoting cell migration and survival (52), the effects of inhibiting ATX expression in adipocytes by SC144 in obesity-associated cancer progression warrant future investigation.

In conclusion, the current study suggests that ATX expression in adipocytes is upregulated by IL-6 family cytokines through the gp130-JAK-STAT3 pathway. The increase of adipocyte-derived ATX leads to elevated plasma ATX and LPA levels and contributes to insulin resistance in diet-induced obesity (Fig. 6). Oral administration of the gp130

![Fig. 6. A regulatory model of ATX expression in adipose tissue and the biological functions of adipocyte-derived ATX in diet-induced obesity. In diet-induced obesity, the IL-6 family cytokines are produced from adipocytes and infiltrated immune cells in adipose tissue. IL-6 family cytokines upregulate ATX expression in adipocytes through the gp130-JAK-STAT3 signaling pathway. The increased production of adipocyte-derived ATX leads to elevated plasma ATX and LPA levels and contributes to insulin resistance in diet-induced obesity (Fig. 6). Oral administration of the gp130 inhibitor SC144 reduces ATX expression and plasma ATX and LPA levels, confirming that gp130 signaling plays an important role in the regulation of ATX expression in adipocytes. Furthermore, blocking gp130 signaling by SC144 has similar effects as inhibiting LPA1/3 signaling by Ki16425, resulting in suppression of adipose tissue inflammation, decrease of plasma FFA levels, and improvement in glucose tolerance and insulin sensitivity in HFD-induced obese mice. It has been reported recently that gp130-mediated adipose tissue lipolysis promotes hepatic steatosis and insulin resistance (49). In this study, our data suggest that ATX-LPA signaling participates in the modulation of lipolysis. Meanwhile, there is evidence to support that ATX/LPA signaling has direct impact to impair glucose homeostasis (12, 40, 50). We propose that the inhibition of ATX expression and decrease of lipolysis both contribute to the improvement of insulin sensitivity and glucose tolerance in SC144-treated obese mice. Oral administration of SC144 has anticancer activity without significant toxicity to normal tissues (51). Our findings in this study reveal a novel activity of SC144 in the treatment of obesity-associated glucose metabolic disorders. Because the ATX-LPA axis has a role in oncogenesis by promoting cell migration and survival (52), the effects of inhibiting ATX expression in adipocytes by SC144 in obesity-associated cancer progression warrant future investigation.](image-url)
inhibitor, SC144, suppressed ATX expression in adipose tissue and improved both glucose tolerance and insulin sensitivity in HFD-fed obese mice. Our study demonstrates an important molecular mechanism of ATX expression regulation in adipocytes and suggests that gp130 is an attractive target for the treatment of obesity-associated glucose metabolic disorders.

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