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Accumulation of CD11c⁺CD163⁺ Adipose Tissue Macrophages through Upregulation of Intracellular 11 β -HSD1 in Human Obesity

Shotaro Nakajima,* Vivien Koh,[†] Ley-Fang Kua,[†] Jimmy So,[‡] Lomanto Davide,[‡] Kee Siang Lim,* Sven Hans Petersen,* Wei-Peng Yong,*[†] Asim Shabbir,[‡] and Koji Kono*^{‡,§,¶}

Adipose tissue (AT) macrophages (ATMs) are key players for regulation of AT homeostasis and obesity-related metabolic disorders. However, the phenotypes of human ATMs and regulatory mechanisms of their polarization have not been clearly described. In this study, we investigated human ATMs in both abdominal visceral AT and s.c. AT and proposed an 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1)–glucocorticoid receptor regulatory axis that might dictate M1/M2 polarization in ATMs. The accumulation of CD11c⁺CD163⁺ ATMs in both visceral AT and s.c. AT of obese individuals was confirmed at the cellular level and was found to be clearly correlated with body mass index and production of reactive oxygen species. Using our in vitro system where human peripheral blood monocytes (hPBMs) were cocultured with Simpson–Golabi–Behmel syndrome adipocytes, M1/M2 polarization was found to be dependent on 11 β -HSD1, an intracellular glucocorticoid reactivating enzyme. Exposure of hPBMs to cortisol-induced expression of CD163 and RU-486, a glucocorticoid receptor antagonist, significantly abrogated CD163 expression through coculture of mature adipocytes with hPBMs. Moreover, 11 β -HSD1 was expressed in crown ATMs in obese AT. Importantly, conditioned medium from coculture of adipocytes with hPBMs enhanced proliferation of human breast cancer MCF7 and MDA-MB-231 cells. In summary, the phenotypic switch of ATMs from M2 to mixed M1/M2 phenotype occurred through differentiation of adipocytes in obese individuals, and upregulation of intracellular 11 β -HSD1 might play a role in the process. *The Journal of Immunology*, 2016, 197: 3735–3745.

Overweight and obese individuals pose global health issues and are associated with increased risks of metabolic diseases, including type 2 diabetes, cardiovascular disease, and certain cancers (1–3). Obesity and related metabolic disorders are associated with a state of chronic, low-grade inflammation in adipose tissue (AT), and AT macrophages (ATMs)

have been postulated as important elements in inflammation (4, 5). Understanding the regulatory mechanisms of ATM polarization can be important in preventing the development of obesity-related metabolic disorders. However, human ATM phenotypes and their polarization mechanisms remain unclear.

There are at least two different phenotypes of ATMs: classically activated proinflammatory ATMs (M1 ATMs) and alternatively activated anti-inflammatory ATMs (M2 ATMs). In nonobese animals, resident M2 ATMs are predominantly observed and contribute to maintenance of AT homeostasis. Alternatively, during obesity, secretions of chemotactic molecules, such as MCP-1 from hypertrophic adipocytes, result in the recruitment of circulating monocytes into AT and their differentiation into an M1 phenotype (6, 7). Indeed, this phenotypic switch from M2 to M1 in AT is a crucial determinant of insulin resistance in obese mice. In mice fed a high-fat diet, a marked increase in the accumulation of F4/80⁺CD11c⁺ ATM-expressing inflammatory genes, as well as the depletion of these F4/80⁺CD11c⁺ cells, would normalize obesity-induced insulin resistance (8, 9). In contrast, other groups reported that this phenotypic switch in human ATMs in obese individuals is fundamentally different from the mouse model. Zeyda et al. (10) and Bourlier et al. (11) reported that human ATMs were of the M2 phenotype—producing proinflammatory mediators. Another study by Wentworth et al. (12) suggested that the percentage of CD11c⁺CD206⁺ ATMs with mixed M1/M2 phenotype increased in obese subjects and was correlated with markers of increased insulin resistance, such as crown-like structures (CLS), and also with the homeostasis model assessment of insulin resistance. Importantly, Becker and colleagues (13) demonstrated that metabolic activation pathways triggered by glucose, insulin, and palmitate also contribute to ATM-specific polarization in humans and mice. Therefore, the

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Abbreviations used in this article: AT, adipose tissue; ATM, AT macrophage; BMI, body mass index; CLS, crown-like structure; CM, conditioned medium; ER, endoplasmic reticulum; FFA, free fatty acid; GR, glucocorticoid receptor; HbA1c, hemoglobin A1c; hPBM, human peripheral blood monocyte; 11 β -HSD, 11 β -hydroxysteroid dehydrogenase; 11 β -HSD1, 11 β -hydroxysteroid dehydrogenase type 1; 11 β -HSD2, 11 β -hydroxysteroid dehydrogenase type 2; qPCR, quantitative real-time PCR; ROS, reactive oxygen species; SAT, s.c. AT; SGBS, Simpson–Golabi–Behmel syndrome; SVF, stromal vascular fraction; TAM, tumor-associated macrophage; VAT, visceral AT; VEGF, vascular endothelial growth factor.

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regulation of ATM polarization is complex, and little is known of the link between human ATM phenotype and metabolic disorders in obese individuals. Further investigations on the phenotype of human ATMs and their functions are needed to develop efficient therapies for obesity and related metabolic diseases.

Several markers of human M1 and M2 ATMs have been reported. Cell surface markers of M1 ATMs include CD11c, CD40, CD86, HLA-DR, and TLR4 (7, 14, 15). CD11c is predominantly expressed by crown ATMs in the proinflammatory state (12). Several groups also reported CD11c as a human M1 marker (16–18). Thus, it is considered to be a suitable marker of human M1 ATMs. Additionally, there are several known cell surface markers of M2 ATMs such as CD163, CD204 (macrophage scavenger receptors), and CD206 (mannose receptor) (7, 14, 15). Komahara et al. (15) reported that CD163 but not CD204 or CD206 was upregulated by treatment with IL-10, which induces M2 polarization in human monocyte-derived macrophages. Interestingly, CD163 is one of the ATM markers associated with the homeostasis model assessment of insulin resistance (19). Based on published evidence, CD163 can be a useful marker to represent human M2 ATMs.

11 β -Hydroxysteroid dehydrogenases (11 β -HSDs) are enzymes that catalyze the interconversion of intracellular glucocorticoid. 11 β -HSDs consist of two isotypes, 11 β -HSD type 1 (11 β -HSD1) and 11 β -HSD type 2 (11 β -HSD2). 11 β -HSD1 is highly expressed in the liver, AT, ovary, and CNS, whereas 11 β -HSD2 is found expressed in the kidney, colon, sweat glands, and placenta (20). 11 β -HSD1 catalyzes the conversion of inactive cortisone to the active cortisol, whereas 11 β -HSD2 catalyzes the reverse reaction (21). It has been reported that both the mRNA level and activity of 11 β -HSD1 correlate with body mass index (BMI) in both human visceral AT (VAT) and s.c. AT (SAT) (22–24). 11 β -HSD1 is expressed not only in adipocytes but also in stromal/vascular compartments of human SAT (25, 26), suggesting that ATMs might also express 11 β -HSD1. Indeed, a positive correlation was observed between the expression of macrophage marker CD68 and 11 β -HSD1 in human SAT (27). Moreover, glucocorticoid is known as a major inducer of CD163 expression in monocytes (28, 29). Taken together, it is likely that the 11 β -HSD1–glucocorticoid receptor (GR) axis may regulate M2 polarization, especially CD163 expression, in monocytes/macrophages in AT in obese individuals.

In the present study, we defined CD11c⁺CD163⁺ ATMs having a mixed M1/M2 phenotype of human ATMs and evaluated their prevalence in both VAT and SAT of obese individuals. Additionally, we successfully established an *in vitro* coculture system to examine 1) the effects of adipocyte differentiation on polarization of human peripheral blood monocytes (hPBMs), and 2) the signaling molecule important for hPBM polarization. Finally, we also investigated the effects of mixed M1/M2 monocytes/macrophages on obesity-associated tumor progression using breast cancer cell lines.

Materials and Methods

Materials

Reagents used in this study included biotin, collagenase, dexamethasone, D-pantothenic acid hemicalcium salt (pantothenate), human insulin, human transferrin, hyaluronidase, hydrocortisone (cortisol), palmitic acid, PMA, RU-486, sodium azide, 1-methyl-3-isobutyl-xanthine, 3,3',5-triiodo-L-thyronine sodium salt (triiodothyronine), and DAPI (Sigma-Aldrich, St. Louis, MO); rosiglitazone (Cayman Chemical, Ann Arbor, MI); allophycocyanin-conjugated human CD14 Ab, FITC-conjugated human CD163 Ab, PE-conjugated human CD11c Ab, and human caveolin Ab (BD Biosciences; San Jose, CA); 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester, Alexa Fluor 488-conjugated anti-mouse Ab, and Alexa Fluor 647-conjugated anti-rabbit Ab (Invitrogen, Carlsbad, CA);

human CD68 Ab (Dako, Glostrup, Denmark); human 11 β -HSD1 Ab (Abcam, Cambridge, U.K.); and β -actin Ab (Cell Signaling Technology, Danvers, MA).

Collection of AT

This study was approved by the Domain-Specific Ethics Board of the National University Hospital of Singapore (DSRB no. 2015/00031). We recruited 7 nonobese subjects (BMI < 30 kg/m²) and 22 obese subjects (BMI \geq 30 kg/m²) from the National University Hospital of Singapore (Table 1). Abdominal VAT (omental AT) and SAT were collected during herniorrhaphy for nonobese subjects and during bariatric surgery for obese subjects after obtaining informed consent. None of the subjects had any clinical symptoms of systemic inflammation.

Isolation of stromal vascular fraction from AT

AT was minced with sterile scissors and digested in HBSS (Life Technologies, Carlsbad, CA) containing 300 U/ml collagenase and 100 U/ml hyaluronidase supplemented with 10% BSA and 20 mM HEPES at 37°C for 60 min. The digested sample was sieved through a 70- μ m filter (BD Biosciences) and centrifuged at 600 \times g for 10 min to extract stromal vascular fraction (SVF) pellets, which contain preadipocytes, endothelial cells, and immune cells, including macrophages. The SVF pellet was incubated with RBC lysis solution (Qiagen, Hilden, Germany) for 8 min and centrifuged at 300 \times g for 10 min. Finally, the pellet was washed with PBS and resuspended in staining buffer containing 3% FBS and 0.05% sodium azide for flow cytometry analysis.

Flow cytometry analysis

SVF cells or hPBMs were stained with Abs specific for PE-conjugated human CD11c, allophycocyanin-conjugated human CD14, and FITC-conjugated human CD163 in the presence of FcR blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany). For detection of intracellular reactive oxygen species (ROS) production, cells were incubated with 1 mM 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester with or without 0.5 μ g/ml PMA for 20 min at 37°C shielded from light. The reaction was stopped by the addition of ice-cold PBS, and cells were stained with allophycocyanin-conjugated human CD14 Ab. The level of ROS production was determined as mean dichlorofluorescein fluorescent intensity of PMA-treated CD14⁺ cells minus mean dichlorofluorescein fluorescence intensity of nontreated CD14⁺ cells. Cells were gated on CD14⁺ cells for macrophage phenotyping using an LSR II flow cytometer (BD Biosciences). The analyses of FACS data were performed using FlowJo software (Tree Star, Ashland, OR).

Immunofluorescence and H&E staining

Paraffin-embedded 5- μ m AT sections fixed in 10% formaldehyde were deparaffinized and rehydrated. For immunofluorescence staining, the sections were then incubated with epitope retrieval solution (Dako) for 20 min at 100°C. After washing with TBST, the sections were treated with blocking solution for 30 min and stained with anti-human CD68 Ab, anti-human caveolin Ab, and/or anti-human 11 β -HSD1 Ab at 4°C overnight. The sections were washed and incubated for 1 h with Alexa Fluor 488-conjugated anti-mouse, Alexa Fluor 555-conjugated anti-rabbit, and Alexa Fluor 647-conjugated anti-rabbit secondary Abs. Nuclei were stained with DAPI for 10 min. Finally, slides were mounted using ProLong Gold antifade reagent (Invitrogen), and images were obtained using the Nikon A1R confocal microscope (Nikon, Tokyo, Japan). CLS were defined as three or more CD68⁺ cells surrounding an adipocyte, and 20 randomly chosen low-power (\times 10) fields were counted by one observer. For H&E staining, deparaffinized and rehydrated AT sections were stained with H&E and then mounted using Eukitt quick-hardening mounting medium (Sigma-Aldrich). Adipocyte areas were measured as 200 cells per AT using ImageJ software.

Cell lines

The human Simpson–Golabi–Behmel syndrome (SGBS) preadipocyte cell line was provided by Dr. Martin Wabitsch (University of Ulm, Ulm, Germany). The human T cell acute lymphoblastic leukemia MOLT-4 cell line was provided by Dr. Takaomi Sanda (National University of Singapore, Singapore). The human acute monocytic leukemia THP-1 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan), the human Hodgkin's disease-derived HDLM-2 cells were from the German Collection of Microorganisms and Cell Culture (Braunschweig, Germany), and the human breast cancer MCF7 and MDA-MB-231 cell lines were from the American Type Culture Collection (Manassas, VA). SGBS cells were maintained in DMEM/F-12 (1:1) (Life

Technologies) supplemented with 10% non-heat-inactivated FBS, 33 μ M biotin, and 17 μ M pantothenate. MOLT-4, THP-1, HDLM-2, MCF7, and MDA-MB-231 cell lines were maintained in RPMI 1640 (Biological Industries, Kibbutz Beit Haemek, Israel) supplemented with 5–10% FBS.

Adipogenic differentiation of SGBS cells

SGBS cells were washed twice with PBS and then cultured in serum-free DMEM/F-12 differentiation medium containing 33 μ M biotin, 17 μ M pantothenate, 0.01 mg/ml human transferrin, 20 nM human insulin, 0.1 μ M cortisol, 0.2 nM triiodothyronine, 25 nM dexamethasone, 250 μ M 1-methyl-3-isobutyl-xanthine, and 2 μ M rosiglitazone. After 4 d, the medium was changed to fresh serum-free medium supplemented with biotin, pantothenate, human transferrin, human insulin, cortisol, and triiodothyronine, and cells were further incubated for several days. The medium was refreshed every 4 d. To quantify lipid accumulation, cells were fixed with 10% formaldehyde and stained with Oil Red O (Sigma-Aldrich).

Coculture of hPBMs with SGBS cells

hPBMs were cocultured directly or indirectly with SGBS cells at various stages of differentiation for 4 d. Direct cocultures were performed by directly adding hPBMs to six-well culture plates containing SGBS cells. Indirect cocultures were performed by adding hPBMs into 0.4- μ m pore size cell culture inserts (Merck Millipore, Jaffrey, NH) and placing them into six-well culture plates containing SGBS cells.

Isolation of monocytes from PBMCs

The use of buffy coats from healthy donors was approved by the Domain-Specific Review Board of the National University Hospital of Singapore (DSRB no. 2015/00031). PBMCs were isolated from buffy coats using Ficoll-Paque (GE Healthcare, Little Chalfont, U.K.). Human monocytes (hPBMs) were isolated using MACS magnetic cell separation systems (Miltenyi Biotec).

Isolation of total RNA

Total RNA was isolated from human AT, human peripheral blood monocytes, and cell lines using TRIzol (Invitrogen) or an RNeasy Mini kit (Qiagen) according to the manufacturers' instructions. RNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE), and the quality was verified by agarose gel electrophoresis.

PCRs

cDNA was synthesized using a HiSenScript RH(–) cDNA synthesis kit (iNtRON Biotechnology, Gyeonggi, South Korea). Semiquantitative PCR was performed on a Veriti 96-well thermal cycler (Applied Biosystems, Carlsbad, CA) using FastStart Taq DNA polymerase and dNTPack (Roche, Basel, Switzerland). Quantitative real-time PCR (qPCR) was performed on an ABI 7500 Fast real-time PCR system (Applied Biosystems) using SYBR Fast qPCR Master mix (Kapa Biosystems, Wilmington, MA). The quantitative PCR data were normalized relative to housekeeping genes, namely GAPDH for monocytes, specificity protein 1 for SGBS cells, and peptidylprolyl isomerase A for AT. Sequences of primers can be provided upon request.

Microarray hybridization and data analysis

Total RNA was isolated as described above. Microarray gene expression analysis was performed using Illumina HT-12 v4 Expression BeadChips (Illumina, San Diego, CA) according to the manufacturer's instructions. Briefly, samples were first amplified, purified, and biotinylated and then hybridized onto HT-12 arrays for 16 h at 56°C. After that, the arrays were washed and subsequently scanned using the Illumina BeadArray Reader. Raw data were exported and analyzed using the GenomeStudio v2011.11 software (Illumina). Differences between the log₂ signal intensities of hPBMs cocultured with 14-d differentiated SGBS adipocytes and hPBMs cocultured with SGBS preadipocytes were calculated to obtain fold-change values indicating regulation in gene expression. The microarray data are available in the Gene Expression Omnibus database (accession no. GSE86492; <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE86492>).

Western blot analysis

SGBS cells and hPBMs were lysed in RIPA buffer (25 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) with a protease inhibitor mixture and a phosphatase inhibitor mixture (Sigma-Aldrich). The cell lysate was mixed with sample buffer containing

50 mM DTT and bromophenol blue and boiled for 5 min. The protein concentration was measured by using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA), and proteins were loaded onto preparative gels (Invitrogen) and transferred to polyvinylidene difluoride membranes. The membranes were incubated with 5% milk blocking solution for 1 h at room temperature, followed by incubation with anti-11 β -HSD1 or anti- β -actin Abs overnight at 4°C. Each membrane was then washed three times with TBST and incubated with HRP-conjugated anti-rabbit Ab for 1 h at room temperature. Immunoreactive proteins were visualized using ECL Prime and/or ECL Select (GE Healthcare).

Small interfering RNA transient transfection

Using RNAiMAX transfection reagent (Invitrogen), SGBS cells were transiently transfected with 11 β -HSD1 small interfering RNA (siRNA) (Mission siRNA, Sigma-Aldrich) or control siRNA (Mission siRNA universal negative control, Sigma-Aldrich). After 72 h, the transfected cells were seeded into each well of a six-well plate and differentiated for 7 d. The expression level of 11 β -HSD1 was confirmed by Western blot analysis.

Cell proliferation assay

A cell proliferation assay was performed using Cell Proliferation ELISA, BrdU (Roche, Basel, Switzerland), according to the manufacturer's instructions. MCF7 cells and MDA-MB-231 cells were seeded at $1-2 \times 10^3$ cells per well in 96-well dishes 24 h before conditioned medium (CM) treatment. CM from hPBMs, 14-d differentiated SGBS adipocytes, or coculture of 14-d differentiated SGBS adipocytes with hPBMs were collected, diluted with 10% FBS culture medium, and filtered to remove floating and apoptotic cells. Finally, MCF7 and MDA-MB-231 cells were treated with CM for up to 4 d and subjected to a proliferation assay.

Statistical analysis

All data are expressed as means \pm SEM. A Spearman correlation test was used to analyze the association in each experiment. A Mann-Whitney *U* test, unpaired *t* test, Student *t* test, or one-way ANOVA was performed to determine statistical significance, and differences were considered to be significant at *p* < 0.05.

Results

Accumulation of CD11c⁺CD163⁺ ATMs in both abdominal VAT and SAT in human obesity

To study the phenotypic switch of human ATMs in obese individuals, SVF cells were isolated from AT and stained with Abs against CD14 (a monocyte/macrophage marker), CD11c (a marker of M1-like ATM), and CD163 (a marker of M2-like ATM) for three-color FACS analysis. In nonobese AT, CD11c[–]CD163⁺ ATMs (AT-resident M2 ATMs) were predominant in both abdominal VAT and SAT (Fig. 1A). However, in obese AT, the percentages of CD11c⁺CD163[–] ATMs (monocyte-like ATMs) and CD11c⁺CD163⁺ ATMs (mixed M1/M2 ATMs) were higher in comparison with nonobese AT (Fig. 1A). A high positive correlation was clearly observed between CD11c⁺CD163⁺ ATMs and BMI in both VAT and SAT (Fig. 1B), and CD11c⁺CD163[–] ATMs were also moderately associated with BMI (Fig. 1C). Alternatively, the percentage of CD11c[–]CD163⁺ ATMs was inversely correlated with BMI in both ATs (Fig. 1D). The percentages of CD11c⁺CD163⁺ and CD11c⁺CD163[–] ATMs but not CD11c[–]CD163⁺ ATMs of total SVF cells were also correlated with BMI in VAT and SAT (Supplemental Fig. 1A), suggesting that CD11c⁺CD163⁺ and CD11c⁺CD163[–] ATMs were overall increased in VAT and SAT in obese subjects. The increased percentage of CD11c⁺CD163⁺ ATMs might be driven by obesity and not by diabetes statuses, because the percentage was positively associated with BMI but not the levels of fasting glucose and hemoglobin A1c (HbA1c) in obese subjects (Supplemental Fig. 1B). Among the diabetes statuses studied, only insulin level was correlated with the percentage of CD11c⁺CD163⁺ ATMs (Supplemental Fig. 1B). Interestingly, the percentage of CD11c⁺CD163⁺ ATMs was significantly higher in obese SAT than in obese VAT (Fig. 1E), and a similar trend was observed in CD11c⁺CD163[–] ATMs (Fig. 1F). The percentage of CD11c[–]CD163⁺

Table I. Clinical characteristics and metabolism parameters of nonobese and obese subjects

	Nonobese (BMI < 30 kg/m ²)	Obese (BMI ≥ 30 kg/m ²)
n (Male/female)	7 (7/0)	22 (7/15)
Age (y)	50.6 ± 7.4 (21–66)	43.0 ± 2.4 (21–61)
Race (Chinese/Indian/Malay/others)	4/1/0/2	5/5/11/1
BMI (kg/m ²)	23.9 ± 0.8 (22.5–28.3)	44.9 ± 1.9 (32.5–62.8)****
Hypertension	2/7	9/22
Type 2 diabetes	0/7	9/22
Fasting glucose (mmol/l)	ND	7.1 ± 0.6 (4.6–13.7)
Fasting insulin (mU/l)	ND	24.0 ± 3.4 (6.3–75.1)
Fasting triglyceride (mmol/l)	ND	1.5 ± 0.1 (0.8–3.4)
HDL cholesterol (mmol/l)	ND	1.2 ± 0.1 (0.7–1.8)
HbA1c (%)	ND	6.6 ± 0.3 (5.1–9.5)

Data are means ± SEM.

*****p* < 0.0001 for lean versus obese.

HDL, high-density lipoprotein.

ATMs was lower in obese SAT than in obese VAT (Fig. 1G). Importantly, note in our comparison analyses between non-obese and obese subjects that there were no female nonobese subjects, whereas >65% of the obese subjects were females. Despite this, no significant differences were found between males and females in all of the parameters studied (data not shown), indicating that gender is not a decisive factor affecting our research findings.

Activated monocytes/macrophages produce ROS that can affect their microenvironment (30, 31). Therefore, we investigated ROS production in ATMs of obese individuals. Production of ROS in ATMs was found to be positively associated with BMI (Fig. 1H). The percentages of CD11c⁺CD163⁺ ATMs and CD11c⁺CD163[−] ATMs were also positively associated with ROS production in ATMs in both ATs (Fig. 1I, 1J). In contrast, inverse correlation was observed between ROS production in ATMs and the percentage

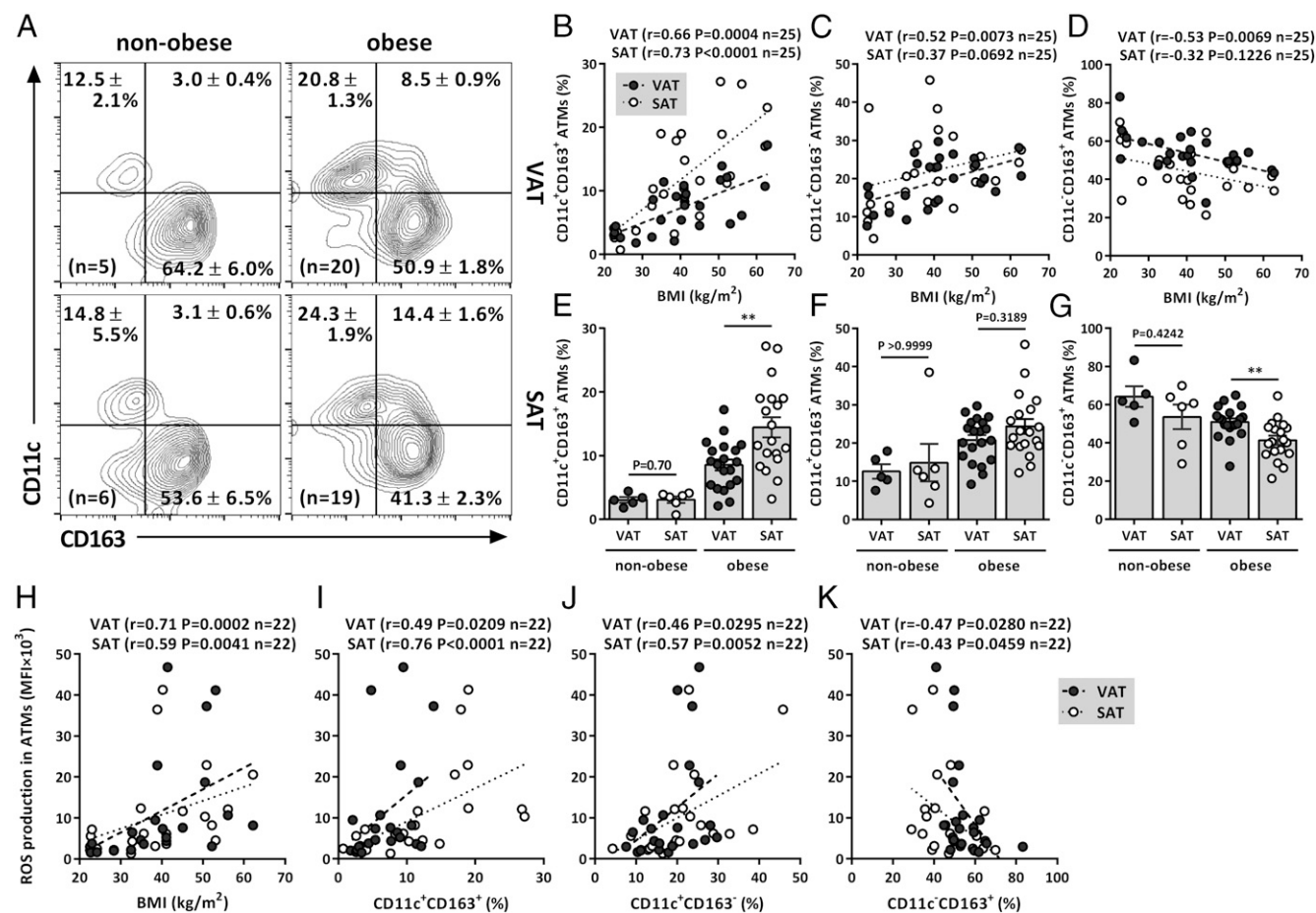


FIGURE 1. Increase in the percentage of CD11c⁺CD163⁺ ATMs in VAT and SAT of obese individuals. (A) Flow cytometry analysis of ATMs in VAT and SAT in nonobese and obese subjects. (B–D) Correlations between the percentage of CD11c⁺CD163⁺ (B), CD11c⁺CD163[−] (C), or CD11c[−]CD163⁺ (D) ATMs and BMI in VAT and SAT. (E–G) Comparison of the percentage of CD11c⁺CD163⁺ (E), CD11c⁺CD163[−] (F), or CD11c[−]CD163⁺ (G) ATMs between VAT and SAT in nonobese (VAT, *n* = 5; SAT, *n* = 6) and obese (VAT, *n* = 20; SAT, *n* = 19) subjects. (H) Correlations between ROS production in ATMs and BMI. (I–K) Correlations between ROS production in ATMs and the percentage of CD11c⁺CD163⁺ (I), CD11c⁺CD163[−] (J), or CD11c[−]CD163⁺ (K) ATMs. ***p* < 0.01.

of CD11c⁺CD163⁺ ATMs in both ATs (Fig. 1K). These data suggest that ATMs, especially CD11c⁺CD163⁺ ATMs and CD11c⁺CD163⁺ ATMs, produce ROS in obese individuals.

Induction of adipocyte hypertrophy and dysfunction in VAT and SAT in obese individuals

We next examined the morphological and structural differences between VAT and SAT in obese individuals. Hypertrophic adipocytes were found in both VAT and SAT in an obese subject (BMI, 50.9 kg/m²) when compared with a nonobese subject (BMI, 22.5 kg/m²) (Fig. 2A). Indeed, hypertrophic adipocytes were observed in obese VAT and SAT, especially in the higher BMI group (BMI ≥ 45) (Fig. 2B). The size of each adipocyte was larger in obese AT than in nonobese AT and was associated with BMI, but there was no significant difference between VAT and SAT in each group (Fig. 2C). We also measured the mRNA levels of adipokines, including adiponectin and leptin. As expected, the mRNA level of adiponectin was lower in obese SAT

than in nonobese SAT (Fig. 2D). However, the level remained unchanged in VAT from nonobese and obese subjects (Fig. 2D). Alternatively, the mRNA level of leptin was elevated more in obese AT than in nonobese AT, as expected, and the increase in leptin was not significant in SAT (Fig. 2D). ATMs aggregate around necrotic adipocytes known as CLS, which is one of the features of obesity (32). As shown in Fig. 2E, the number of CLS increased in obese VAT and SAT and was correlated with BMI. However, there was no significant difference between VAT and SAT within the group (Fig. 2E). Increased adipocyte size and the number of CLS were also driven by obesity but not by diabetes statuses because only BMI, but not fasting glucose, fasting insulin, or HbA1c, was positively associated with these observations in VAT and SAT in obese subjects (Supplemental Fig. 1C).

Our data suggest that although adipocyte hypertrophy and dysfunction occur in obesity, there are not many significant differences between VAT and SAT.

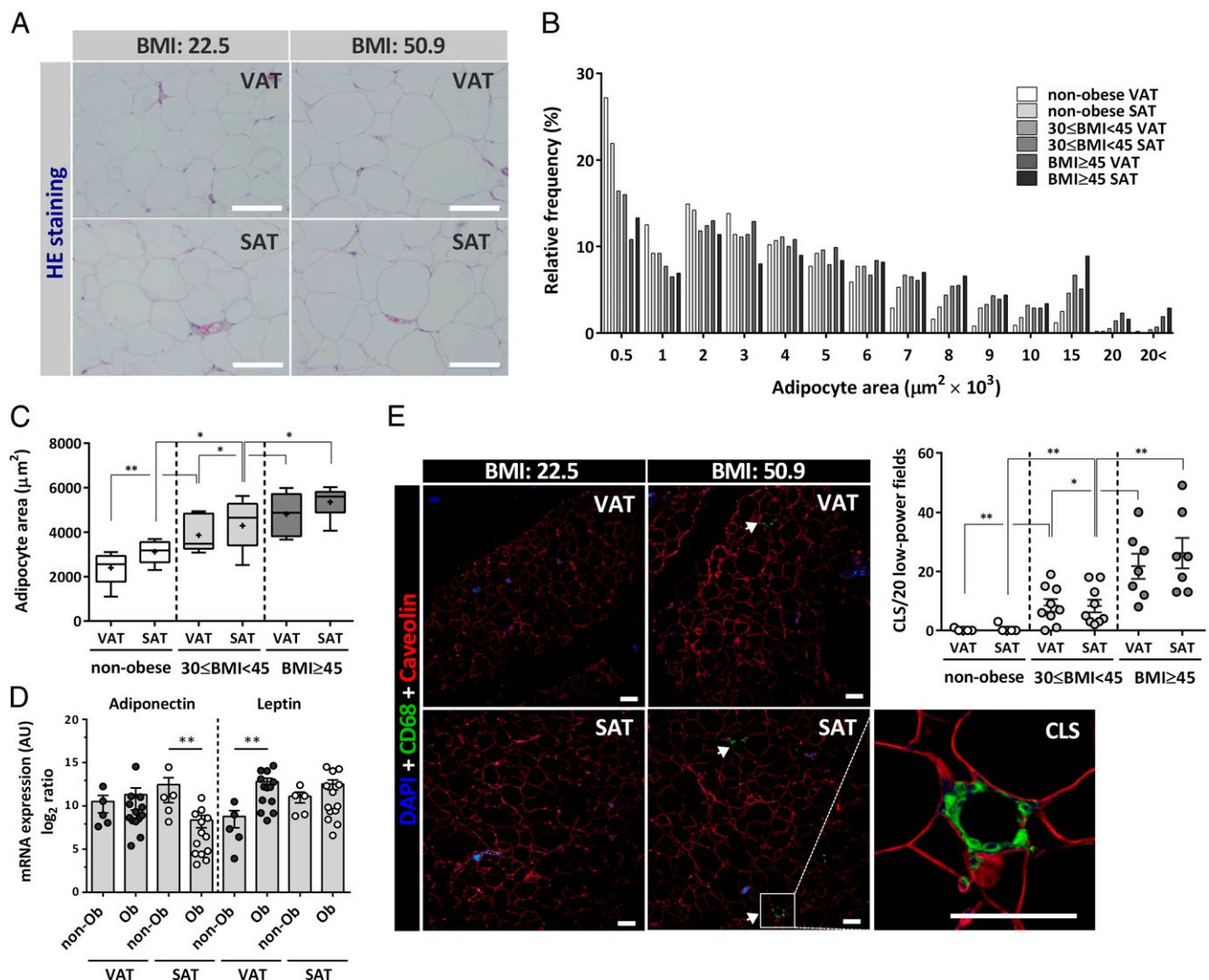


FIGURE 2. Adipocyte hypertrophy and dysfunction in VAT and SAT in obese individuals. **(A)** H&E staining of VAT and SAT in a nonobese (BMI, 22.5 kg/m²) and an obese (BMI, 50.9 kg/m²) subject. Scale bars, 100 μm; original magnification ×20. **(B)** and **(C)** Relative frequency (**B**) and box plots (**C**) of adipocyte size (area per adipocyte) in VAT and SAT in nonobese ($n = 5$), obese ($30 \leq \text{BMI} < 45$) ($n = 9$), and very obese ($\text{BMI} \geq 45$) ($n = 7$) subjects. The lines inside the box plot represent the median size, and plus signs indicate the average size. **(D)** qPCR analysis of adipocyte function markers adiponectin and leptin in VAT and SAT in nonobese ($n = 5$) and obese ($n = 13$) subjects. **(E)** Immunofluorescence staining of CD68 (green), caveolin (red), and DAPI (blue) in VAT and SAT in a nonobese (BMI, 22.5 kg/m²) and an obese (BMI, 50.9 kg/m²) subject. Arrows indicate CLS. Scale bars, 100 μm (left). The numbers of CLS in VAT and SAT in nonobese ($n = 5$), obese ($30 \leq \text{BMI} < 45$) ($n = 9$), and very obese ($\text{BMI} \geq 45$) ($n = 7$) subjects (right) are shown. * $p < 0.05$, ** $p < 0.01$. AU, arbitrary unit; Non-Ob, nonobese; Ob, obese.

Induction of M1 and M2 markers and ROS production in hPBMs when cocultured with human SGBS adipocytes

To next examine whether adipocyte hypertrophy and dysfunction in obese individuals affects polarization of monocytes/macrophages, we established an *in vitro* coculture system using hPBMs and human SGBS preadipocytes (33). Accumulation of lipid droplets and the gene expression levels of adipose function and differentiation markers were elevated in SGBS cells after adipogenic differentiation for 7 d, with >90% of the cells having differentiated to mature adipocytes after 14 d (Fig. 3A, 3B). To examine the effect of adipogenic differentiation on the polarization of hPBMs, hPBMs were cocultured with or without SGBS cells at various stages of differentiation (undifferentiated preadipocytes, 7- or 14-d differentiated adipocytes)

for 4 d, and the surface expressions of CD11c and CD163 in hPBMs were assessed using FACS. Most hPBMs expressed CD11c but not CD163 at the basal level (Fig. 3C, left). Four days of coculturing with SGBS adipocytes resulted in an upregulated expression of CD163 in hPBMs, and most of the cells exhibited a CD11c⁺CD163⁺ phenotype (Fig. 3C, left). Importantly, the expression level of CD163 was significantly higher in hPBMs cocultured with 14-d differentiated adipocytes (mature adipocytes) than those with 7 d differentiation (Fig. 3C, right), suggesting that adipogenic differentiation affects polarization of hPBMs. Moreover, a similar outcome was observed in the indirect coculture system (Fig. 3D), indicating that released factors from adipocytes, but not cell–cell contact between adipocytes and hPBMs, are crucial for the polarization of hPBMs.

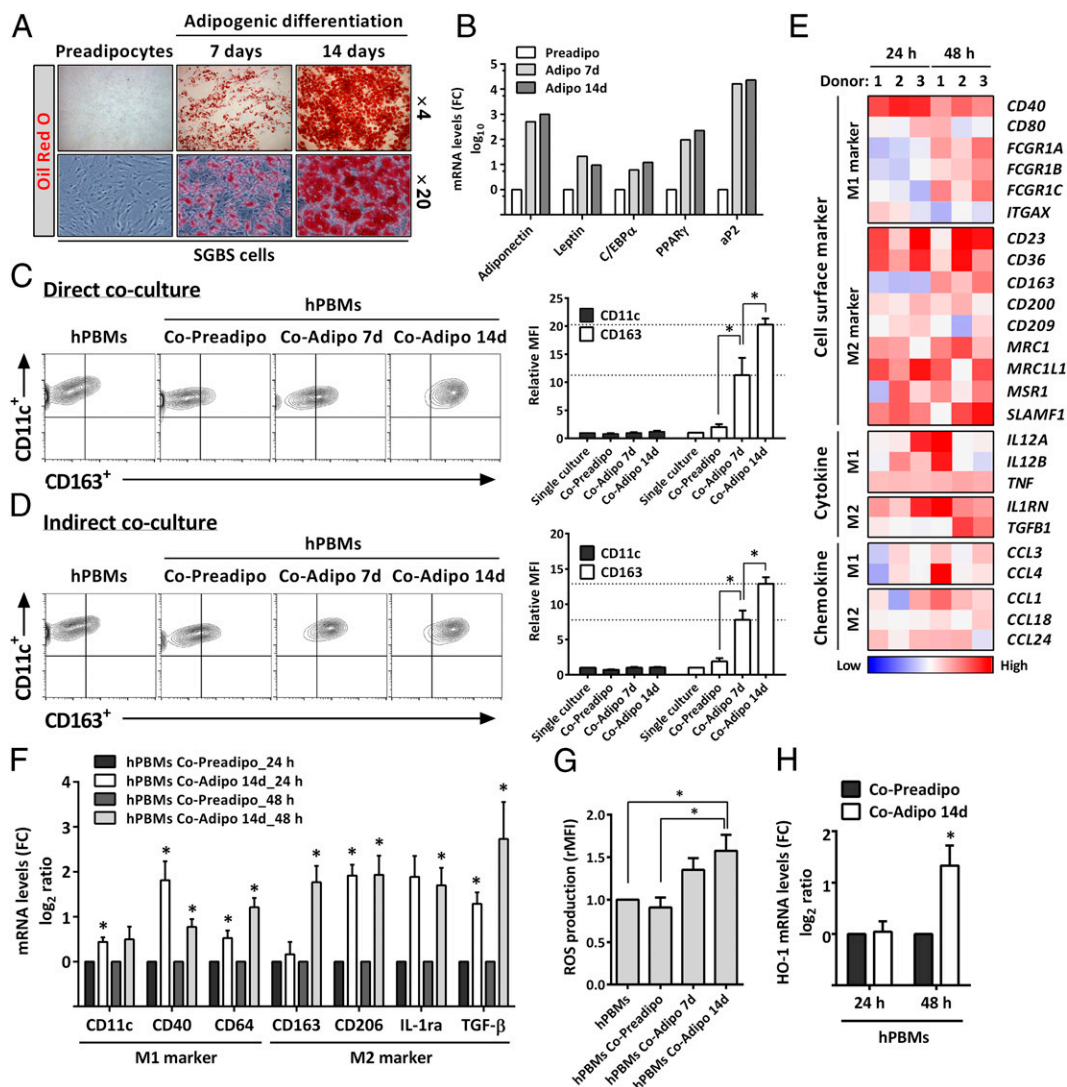


FIGURE 3. Induced expression of M1 and M2 markers in hPBMs when cocultured with SGBS adipocytes. **(A)** Oil Red O staining of undifferentiated or differentiated SGBS cells. **(B)** qPCR analysis of adipocyte differentiation and function markers in SGBS cells during adipogenic differentiation. Data are representative of three independent experiments. **(C and D)** Flow cytometry analysis of hPBMs cocultured directly (C) or indirectly (D) in the presence or absence of SGBS cells at various stages of differentiation (preadipocytes, 7-d differentiated adipocytes, and 14-d differentiated adipocytes) for 4 d ($n = 3$). The flow plots were gated on CD14⁺ cells. Co-Adipo 7d, coculture with 7-d differentiated SGBS adipocytes; Co-Adipo 14d, coculture with 14-d differentiated SGBS adipocytes; Co-Preadipo, coculture with SGBS preadipocytes. **(E)** Heat map showing changes in gene expression of M1 and M2 markers in hPBMs cocultured for 24 and 48 h. hPBMs were derived from three healthy donors (donors 1, 2, and 3) and cocultured with SGBS cells (preadipocytes) or SGBS cells differentiated for 14 d (adipocytes). Expression represents fold differences between values of hPBMs cocultured with SGBS adipocytes and hPBMs cocultured with SGBS preadipocytes. Red indicates high expression (upregulation); blue indicates low expression (downregulation). **(F)** qPCR analysis of selected representative M1 (CD11c, CD40, and CD64) and M2 (CD163, CD206, IL-1ra, and TGF- β) markers in hPBMs cocultured with SGBS preadipocytes or 14-d differentiated SGBS adipocytes for 24 and 48 h ($n = 4$). **(G)** ROS production in hPBMs directly cocultured with or without SGBS preadipocytes, 7-d differentiated SGBS adipocytes, or 14-d differentiated SGBS adipocytes ($n = 3$). **(H)** qPCR of HO-1 in hPBMs cocultured with SGBS preadipocytes or 14-d differentiated SGBS adipocytes for 24 and 48 h ($n = 4$). * $p < 0.05$.

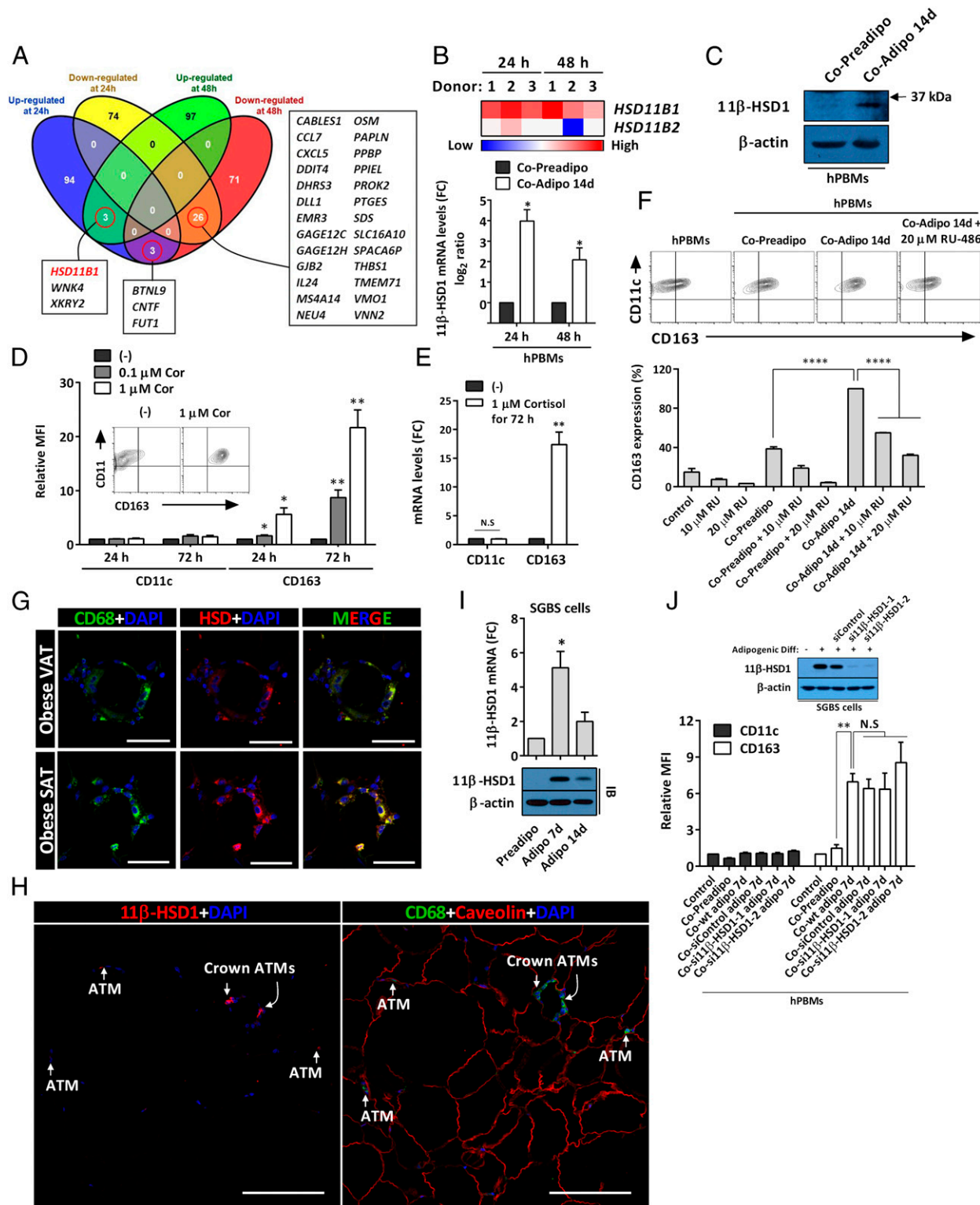


FIGURE 4. Induced CD163 expression in hPBMs through activation of 11β-HSD1–GR axis. **(A)** Venn diagram of the top 100 up- and downregulated genes in hPBMs cocultured with 14-d differentiated SGBS adipocytes versus hPBMs cocultured with SGBS preadipocytes for 24 and 48 h. **(B)** Heat map showing fold changes in expression of 11β-HSD1 (*HSD11B1*) and 11β-HSD2 (*HSD11B2*) in hPBMs cocultured with 14-d differentiated SGBS adipocytes compared with hPBMs cocultured with SGBS preadipocytes. **(C)** qPCR analysis of 11β-HSD1 in hPBMs cocultured with SGBS preadipocytes or 14-d differentiated SGBS adipocytes for 24 and 48 h (bottom) ($n = 4$). Co-Adipo 14d, coculture with 14-d differentiated SGBS adipocytes; Co-Preadipo, coculture with SGBS preadipocytes. **(D)** Western blot analysis of 11β-HSD1 in hPBMs cocultured with SGBS preadipocytes or 14-d differentiated SGBS adipocytes for 36 h. Data are representative of three independent experiments. **(E)** Flow cytometry (D) and qPCR (E) analyses of CD11c and CD163 in hPBMs by treatment with cortisol for 24 and 72 h ($n = 3$). The flow plots were gated on CD14⁺ cells. **(F)** Flow cytometry analysis of CD11c and CD163 in hPBMs cocultured with or without SGBS preadipocytes or 14-d differentiated SGBS adipocytes in the absence or presence of RU-486 (RU) for 4 d ($n = 3$). The flow plots were gated on CD14⁺ cells. Co-Adipo 7d, coculture with 7-d differentiated SGBS adipocytes; Co-Adipo 14d, coculture with 14-d differentiated SGBS adipocytes; Co-Preadipo, coculture with SGBS preadipocytes. **(G)** Representative immunofluorescence staining of CD68 (green), 11β-HSD1 (red), and DAPI (blue) in obese VAT and SAT. Colocalizations of 11β-HSD1 and CD68 are represented in yellow in the merged image. Scale (Figure legend continues)

Microarray analysis revealed that most of the M1 and M2 markers were upregulated in hPBMs cocultured with 14-d differentiated SGBS adipocytes (Fig. 3E). Similarly, qPCR of the selected genes confirmed upregulation of the M1 and M2 markers (Fig. 3F). The mRNA expressions of monocyte to macrophage differentiation-associated 2 (Supplemental Table I) and mature macrophage marker CD64 (Fig. 3E, 3F) were upregulated in hPBMs cocultured with SGBS adipocytes, suggesting that the differentiation of hPBMs might have occurred during the coculture with SGBS adipocytes. Our results also indicated that ROS production was significantly increased in hPBMs cocultured with SGBS adipocytes (Fig. 3G), and that the expression of an oxidative stress inducible gene, HO-1, was significantly elevated in hPBMs after coculturing with SGBS adipocytes for 48 h (Fig. 3H).

Cytokines and chemokines are known to be important factors for the regulation of M1/M2 polarization in monocytes/macrophages (34, 35). We next examined the gene expression levels of cytokines, chemokines, and signaling molecules regulating these expressions, including NF- κ B, MAPK, as well as STAT signaling, in SGBS cells during adipogenic differentiation. Expression of genes representing adipocyte function and differentiation markers, including *LEP* (leptin), *ADIPOQ* (adiponectin), *APOE* (apolipoprotein E), *FABP4* (adipocyte protein 2), *LPL* (lipoprotein lipase), *LIPE* (lipase E), *RBP4* (retinol-binding protein 4), *PPARG* (peroxisome proliferator-activated receptor γ), and *CEBPA* (C/EBP α), markedly increased during adipogenic differentiation (Supplemental Fig. 2A). However, the expression of inflammatory cytokines and chemokines, such as *IL6* (IL-6), *IL8* (IL-8), and *CCL2* (MCP-1), were significantly downregulated in adipocytes compared with preadipocytes (Supplemental Fig. 2A). The expressions of Th2 cytokines, including IL-4 and IL-13, which trigger alternative M2 polarization of macrophages (35), were also not induced in SGBS adipocytes (Supplemental Fig. 2B), suggesting that these representative cytokines and chemokines might not be involved in the regulation of differentiation and polarization of hPBMs in our system.

Our microarray data showed that the expression of M2 markers was more significantly increased in hPBMs cocultured with SGBS adipocytes than those of M1 markers. Previous reports suggested that endoplasmic reticulum (ER) stress is a key regulator of alternative activation triggering human M2 macrophage differentiation (36, 37). Indeed, our data showed that the expressions of ER stress markers were upregulated in hPBMs cocultured with SGBS adipocytes when compared with hPBMs cocultured with SGBS preadipocytes (Supplemental Fig. 3A). Based on previous reports and our data, ER stress might be involved in the induction of M2 polarization of hPBMs induced by coculturing with adipocytes.

Our results collectively suggest that adipocyte differentiation induces polarization of hPBMs to a mixed M1/M2 phenotype.

Upregulation of CD163 expression in hPBMs cocultured with SGBS adipocytes through upregulation of intracellular 11 β -HSD1 and activation of GR

The top 100 most up- and downregulated genes in hPBMs cocultured with SGBS adipocytes were retrieved from our microarray data (Supplemental Table I). Comparative analysis revealed that three

genes were upregulated at both 24 and 48 h, three genes were upregulated at 24 h and downregulated at 48 h, and 26 genes were downregulated at both 24 and 48 h (Fig. 4A). Among these genes, *HSD11B1* (11 β -HSD1) was found to be highly ranked with significant upregulation. Hence, we decided to focus on 11 β -HSD1 for further experiments.

Fig. 4B shows that the expression of 11 β -HSD1 but not 11 β -HSD2 was upregulated in hPBMs cocultured with SGBS adipocytes from both microarray and qPCR analyses. Gene expression results were similarly associated with elevation of the 11 β -HSD1 protein (Fig. 4C). 11 β -HSD1 converts intracellular cortisone to cortisol, which activates GR and downstream signaling (38). Our in vitro model confirmed that exposure of hPBMs with cortisol markedly induced its surface expression of CD163 in a dose- and time-dependent manner (Fig. 4D) and at the transcriptional level (Fig. 4E). To test whether activation of GR is involved in the upregulation of CD163 in hPBMs, hPBMs were cocultured with SGBS preadipocytes or SGBS adipocytes in the absence or presence of GR antagonist RU-486 and then analyzed by FACS. CD163 expression in hPBMs when cocultured with SGBS adipocytes was abrogated by the treatment with RU-486 in a dose-dependent manner (Fig. 4F), indicating that the activation of GR regulates adipogenic differentiation-induced expression of CD163 in hPBMs, which might be regulated by the upregulation of intracellular 11 β -HSD1. We further examined the expression of 11 β -HSD1 in ATMs in human obese AT. Immunofluorescence staining of AT revealed that 11 β -HSD1 was expressed in crown ATMs in obese VAT and SAT (Fig. 4G), and the expression is higher in crown ATMs compared with noncrown ATMs (Fig. 4H). As the number of CLS is very limited in nonobese AT (Fig. 2E), the expression level of 11 β -HSD1 in ATMs might be upregulated in obese individuals.

It has been known that 11 β -HSD1 is also expressed in adipocytes (25, 26, 39). Indeed, adipogenic differentiation markedly upregulated the gene and protein expressions of 11 β -HSD1 in SGBS cells (Fig. 4I). Therefore, we tested whether cortisol produced in SGBS adipocytes is involved in the induction of CD163 expression in hPBMs. We first measured the concentration of cortisol in CM of SGBS adipocytes. However, we did not detect any cortisol in CM of SGBS adipocytes even after 14 d of differentiation (S. Nakajima, V. Koh, L.F. Kua, and K. Kono, unpublished data). We next examined the effect of 11 β -HSD1 expression in SGBS cells on the polarization of hPBMs. SGBS cells were transiently transfected with 11 β -HSD1 siRNA or control siRNA and allowed to differentiate to the adipogenic state for 7 d (Fig. 4J). After differentiation, SGBS cells were cocultured with hPBMs, and the surface expressions of CD11c and CD163 in hPBMs were assessed. Coculturing with wild-type SGBS adipocytes markedly induced CD163 expression in hPBMs, and the expression persisted in the coculture with 11 β -HSD1 knockdown SGBS adipocytes (Fig. 4J), implying that 11 β -HSD1-mediated production of cortisol in SGBS adipocytes does not affect the polarization of hPBMs.

Taken together, induction of 11 β -HSD1 within ATMs might increase the concentration of intracellular cortisol, which induces CD163 expression in obese individuals.

bars, 100 μ m. (H) Representative immunofluorescence staining of serial sections in obese VAT with 11 β -HSD1 (red) and DAPI (blue) (left), and CD68 (green), caveolin (red), and DAPI (blue) (right). Scale bars, 100 μ m. (I) qPCR and Western blot (IB) analyses of 11 β -HSD1 in SGBS cells during adipogenic differentiation ($n = 3$). Western blotting data are representative of three independent experiments. (J) Knockdown of 11 β -HSD1 expression in SGBS cells during adipogenic differentiation for 7 d. Data are representative of three independent experiments (top). Flow cytometry analysis of hPBMs cocultured with or without wild-type SGBS cells, control siRNA-transfected SGBS cells, or 11 β -HSD1 siRNA-transfected SGBS cells at 7-d adipogenic differentiation ($n = 3$) (bottom). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

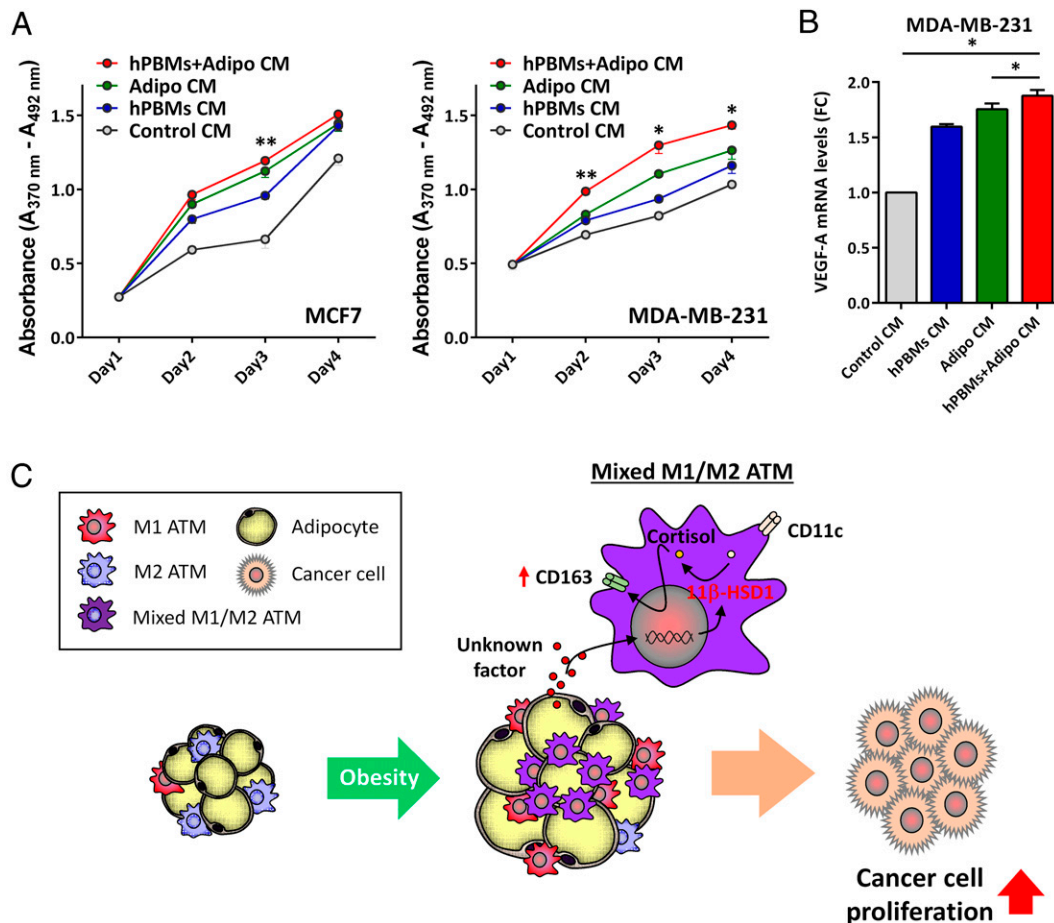


FIGURE 5. Increased breast cancer cell proliferation using CM from coculture of adipocytes with hPBMs. **(A)** Cell proliferation assay of MCF7 and MDA-MB-231 cell lines treated with CM from control (gray), hPBMs (blue), SGBS adipocytes (green), or cocultured SGBS adipocytes with hPBMs (red) for 4 d. Data are representative of three independent experiments. **(B)** qPCR analysis of VEGF-A in MDA-MB-231 cell line treated with each CM for 48 h. Data are representative of three independent experiments. **(C)** Schematic representation of our experimental conclusions. * $p < 0.05$, ** $p < 0.01$.

Enhancement of adipocyte-induced breast cancer cell proliferation by mixed M1/M2 phenotype hPBMs

Obesity might be associated with breast cancer progression (40, 41). We therefore examined whether mixed M1/M2 phenotype monocytes/macrophages are related to breast cancer progression. Exposure of MCF7 and MDA-MB-231 cells to CM from SGBS adipocytes promoted their proliferation. CM from coculture of adipocytes with hPBMs further enhanced the proliferation (Fig. 5A), suggesting that mixed M1/M2 phenotype monocytes/macrophages might contribute to obesity-associated tumor growth. Moreover, CM from coculture of adipocytes with hPBMs also enhanced the mRNA expression of vascular endothelial growth factor (VEGF)-A in MDA-MB-231 cells (Fig. 5B). These data suggest that obesity-induced mixed M1/M2 ATMs might be involved in obesity-associated tumor growth and angiogenesis through enhancement of cancer cell proliferation and production of VEGF in cancer cells.

Discussion

In this study, we report that CD11c⁺CD163⁺ ATMs of a mixed M1/M2 phenotype accumulated in obese individuals, and both the upregulation of intracellular 11β-HSD1 and activation of GR in ATMs are important for regulation of the polarization of ATMs. Additionally, CD11c⁺CD163⁺ ATMs might contribute to obesity-associated cancer through promotion of cancer cell growth and angiogenesis (Fig. 5C).

The expression of 11β-HSD1 is induced in human monocytes upon differentiation and M2 polarization (42, 43). Our present data showed that the expression of 11β-HSD1 was induced in hPBMs cocultured with adipocytes, which was involved in the expression of the M2 marker CD163 (Fig. 4). According to a published dataset in the National Center for Biotechnology Information GEO database (dataset record GDS2429), 11β-HSD1 but not 11β-HSD2 was upregulated in both LPS plus IFN-γ-induced human M1-activated macrophages and IL-4-induced human M2-activated macrophages. Moreover, in a murine macrophage J774.1 cell line, the mRNA expression and enzymatic activity of 11β-HSD1 were augmented by LPS stimulation, and pharmacological inhibition of 11β-HSD1 markedly suppressed LPS-induced production of inflammatory cytokines such as IL-1β, TNF-α, and MCP-1 (44), suggesting that 11β-HSD1 regulates not only M2 polarization but also M1 polarization. Immunofluorescence staining of obese AT showed that 11β-HSD1 was highly expressed in crown ATMs compared with noncrown ATMs (Fig. 4H). A previous report suggested that crown ATMs had a mixed M1/M2 phenotype that expressed CD11c and another M2 marker, CD206 (12). Therefore, 11β-HSD1 might be a general marker of adipocyte-induced polarization of ATMs.

Whereas cortisol levels in blood are mainly controlled by the hypothalamic–pituitary–adrenal axis, intracellular cortisol levels within each tissue are regulated by 11β-HSD1 (45). AT has been known as a glucocorticoid target tissue, which expresses 11β-HSD1

and GRs. 11 β -HSD1 is known to be expressed in adipocytes, where it contributes to adipogenic differentiation by converting cortisone to cortisol (46, 47). Cortisol release from AT by 11 β -HSD1 induction in humans has been previously reported (48), suggesting that released cortisol from adipocytes might affect the differentiation and function of other cells, including macrophages, in AT. However, at least in our in vitro system, cortisol from adipocytes was not involved in the polarization of hPBMs because the induction of CD163 expression in hPBMs was not abrogated when hPBMs were cocultured with 11 β -HSD1 knockdown SGBS adipocytes (Fig. 4J). Crown ATMs also expressed 11 β -HSD1 in obese subjects (Fig. 4G, 4H), implying that intracellular cortisol regulated by 11 β -HSD1 has critical roles for polarization and/or function of ATMs in human obesity.

We proposed from our results that ER stress might be involved in alternative M2 activation of hPBMs when the cells were cocultured with SGBS adipocytes. An earlier report suggested that induction of ER stress led to the expression of several M2 markers, including CD36, CD163, and CD206, through activation of c-Jun N-terminal kinase in human peripheral monocytes/macrophages (36). Similarly, our microarray analysis also showed that M2 markers such as *CD36*, *CD163*, and *MRC1* (CD206) (Fig. 3E) and ER stress markers such as *HSPA5* (78-kDa glucose-regulated protein), *HYOU1* (oxygen-regulated protein 150 kDa), and *DDIT3* (C/EBP-homologous protein) were all upregulated in hPBMs cocultured with SGBS adipocytes (Supplemental Fig. 3A). The release of free fatty acids (FFAs) (49) and palmitic acid, a representative FFA having potential to trigger ER stress in human monocytes (50), from hypertrophic adipocytes also suggested that a possible mechanism underlying induction of ER stress and M2 polarization in hPBMs might involve FFAs. We therefore examined whether treatment with palmitic acid affects surface expression of CD11c and CD163 in hPBMs. However, exposure of hPBMs to palmitic acid did not affect the expression of both CD11c and CD163 (Supplemental Fig. 3B), indicating that FFAs might not play a role in the mechanism.

It is currently unknown what factors from adipocytes may induce differentiation and polarization of hPBMs. Our data suggest that the representative pro- and anti-inflammatory cytokines and chemokines such as IL-4, IL-6, IL-8, IL-13, and MCP-1 might not be involved in the induction of M1/M2 polarization of hPBMs in our system (Supplemental Fig. 2). Another candidate is leptin. Our present data showed that the expression of leptin in AT was elevated in obese individuals (Fig. 2D) and that adipogenic differentiation induced leptin expression in SGBS cells (Fig. 3B). Wabitsch et al. (51) also observed a significant increase of leptin release into the CM after adipogenic differentiation of SGBS cells. Gambero and colleagues (52) reported that human monocytes exposed to leptin expressed surface markers of M2 phenotype rather than M1 phenotype. We are currently conducting experiments to elucidate the link between leptin and induction of 11 β -HSD1 in ATMs during obesity.

BMI is associated with an increased frequency of many cancers (2, 3). Particularly, obesity is a significant risk factor for breast cancer in postmenopausal women (40, 41). Even in premenopausal women, the prevalence of estrogen receptor-negative tumor is increased with obesity (53, 54), and some reports have focused on the role of ATMs in obesity-related cancer (55, 56). Indeed, Wagner et al. (57) showed that accumulation of macrophages in inflammatory tumor-associated AT stimulated tumor growth and angiogenesis. Moreover, Arendt et al. (58) further demonstrated that obesity leads to the recruitment of inflammatory macrophages in AT, and that these macrophages secreted CXCL12, which acted on blood vessels to enhance angiogenesis. Gene expression profiles

of human ATMs described in this study showed similarities with those reported in human tumor-associated macrophages (TAMs) (59–61). Besides enhancing angiogenesis, invasion, and metastasis of tumor through production of VEGF, IL-8, and epidermal growth factor, TAMs also produce IL-10, TGF- β , programmed death receptor ligand 1, arginase-1, and PGE₂, which induce Treg infiltration to tumor tissues and suppress the activity of cytotoxic T cells and NK cells at the site of the tumor (34, 62). Our data showed that hPBMs cocultured with mature adipocytes expressed several M2 markers that are also expressed in TAMs. Therefore, ATMs in obese tissues might be involved in tumor progression.

In conclusion, our study indicates that a mixed M1/M2 phenotype of CD11c⁺CD163⁺ ATMs accumulated in obese individuals, and that both the upregulation of intracellular 11 β -HSD1 and activation of GR in ATMs are important for regulating polarization of ATMs. Prevention of polarization and accumulation of CD11c⁺CD163⁺ ATMs can act as novel therapeutic strategies against obesity-related diseases, and the 11 β -HSD1–GR regulatory axis might be a potential target in such therapies.

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Disclosures

The authors have no financial conflicts of interest.

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