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Adipose-specific Monocyte Chemotactic Protein-1 Deficiency Reduces Pulmonary Metastasis of Lewis Lung Carcinoma in Mice

SNEHA SUNDARAM and LIN YAN

U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND, U.S.A.

Abstract. *Aim: Monocyte chemotactic protein-1 (MCP1) is a potent adipokine. This study tested the hypothesis that adipose-produced MCP1 contributes to metastasis. Materials and Methods: In a spontaneous metastasis model of Lewis lung carcinoma (LLC), male adipose MCP1-deficient ($Mcp1^{-/-}$) and wild-type (WT) mice were fed the AIN93G diet or a high-fat diet (HFD) for 11 weeks. Lung metastasis from a subcutaneous tumor was the primary endpoint. Results: The adipose expression of MCP1 was lower in $Mcp1^{-/-}$ mice than in WT controls. The HFD increased the number of lung metastases in WT mice. The number of metastasis was significantly lower in the HFD-fed $Mcp1^{-/-}$ mice than in the HFD-fed WT mice. Compared to the WT mice, adipose MCP1 deficiency lowered plasma concentrations of insulin, proinflammatory adipokines (leptin, plasminogen activator inhibitor-1, and resistin), and angiogenic markers (vascular endothelial growth factor, hepatocyte growth factor, and angiopoietin-2). Conclusion: Adipose MCP1 deficiency attenuates HFD-enhanced pulmonary metastasis of LLC.*

Monocyte chemotactic protein-1 (MCP1), a key member of the chemokine family, is a potent chemotactic factor for monocyte and macrophage infiltration to the site of inflammation during tissue injury and infection (1). Studies have shown that MCP1 participates in pathological processes including cancer and that elevated expression of MCP1 has a prognostic value in clinical practice (2-4). For example, high levels of MCP1 are related to poor outcome and short disease-free survival in patients with breast (2), prostate (3), and colorectal (4) cancer.

Recent decades have seen marked changes in lifestyle that have resulted in an increase in obesity. Visceral adipose accumulation is a strong indicator of detrimental health outcomes (5, 6) because of its active participation in the production of many proinflammatory cytokines including MCP1. Body mass index and body adiposity are positively correlated with adipose MCP1 expression (7). Compared to patients with breast cancer with normal body weights, those who are obese are at a greater risk of cancer recurrence with a shorter disease-free interval (8, 9). Patients with prostate cancer who are obese are more likely to have recurrence after radical prostatectomy (10, 11). In laboratory rodents, adipose accumulation and inflammation are hallmarks of diet-induced obesity. For example, the fat mass of mice fed a high-fat diet (HFD) is positively correlated with concentrations of MCP1 in adipose tissue and plasma (12). Furthermore, diet-induced obesity leads to metabolic disturbance [*e.g.* elevation in insulin (13), resistin (14, 15), and leptin (16)] and angiogenesis [*e.g.* increased expression of angiogenic factors including vascular endothelial growth factor (VEGF) (17, 18) and hepatocyte growth factor (HGF) (19, 20)]. These pathophysiological changes may contribute to diet-enhanced primary tumorigenesis (21-23) and metastasis (17, 21, 24).

The spread of malignant cells from a primary tumor to distant organs is a devastating aspect of cancer. We found that HFD increases body adiposity, which in turn increases the concentration of MCP1 in adipose tissue (25) and plasma (17, 25, 26) and enhances metastasis in mice (17, 25, 26). A reduction in body adiposity by experimental means reduced the plasma concentration of MCP1 in non-tumor bearing mice (16) and mice with metastatic lesions (26). The role of MCP1 in enhancing metastasis is supported by findings showing that whole-body knockout of MCP1 reduces diet-enhanced metastasis (17). However, the specific impact of adipose-derived MCP1 is not clear.

Correspondence to: Lin Yan, Ph.D., USDA, ARS, Grand Forks Human Nutrition Research Center, 2420 2nd Avenue North, Grand Forks, ND 58202, U.S.A. Tel: +1 7017958499, Fax: +1 7017958220, e-mail: lin.yan@ars.usda.gov

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In order to investigate the role of adipose-derived MCP1 in malignant progression, we hypothesized that adipose-derived MCP1 contributes to metastasis. The objective of this study was to test this hypothesis by examining spontaneous pulmonary metastasis of Lewis lung carcinoma (LLC) in adipose-specific MCP1-deficient mice fed a high-fat, obesogenic diet.

Materials and Methods

Animals and diets. The Grand Forks Human Nutrition Research Center in-house breeding program provided transgenic male adipose-specific *Mcp1* knockdown mice (defined as *Mcp1*$^{-/-}$) for this study. The breeders were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Adipose-specific *Mcp1* knockdown was achieved by breeding female C57BL/6 mice (*Mcp1*$^{fl/fl}$) carrying flanked loxP sites (both alleles of exons 2-3 of *Mcp1* gene on chromosome 11) with male *Mcp1*$^{fl/fl}$ C57BL/6 mice expressing the Cre recombinase (*Adipoq-Cre*$^{+}$) driven by the adiponectin promoter (*Mcp1*$^{fl/fl}$/*Adipoq-Cre*$^{+}$). Male mice carrying the two floxed *Mcp1* alleles and positive for Cre expression (*Mcp1*$^{fl/fl}$/*Adipoq-Cre*$^{+}$) were designated as knockdown mice *Mcp1*$^{-/-}$, whereas male littermates negative for Cre expression (*Mcp1*$^{fl/fl}$/*Adipoq-Cre*$^{-}$) served as wild-type (WT) controls. Mice were housed in a pathogen-free room on a 12:12-hour light/dark cycle with a temperature of 22±1˚C. The control AIN93G diet (27) and a modified AIN93G diet containing 45% energy from soybean oil (HFD) were used in this study (Table I). Gross energy of each diet (Table I) was quantified using an oxygen bomb calorimeter (Model 6200; Parr Instrument, Moline, IL, USA). Both diets (powder form) were kept at −20˚C; fresh diets were provided to mice every other day.

LLC cell line. The LLC cell line, a variant that metastasizes to the lungs (28), was obtained from Dr. Pnina Brodt, McGill University, Montreal, Quebec, Canada. The cells were cultured in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum and maintained in a humidified atmosphere of 5% CO_2 in air at 37˚C. Cells used for this study were *in vivo*-selected once (29). Cells were free of mycoplasma based on Hoechst DNA staining and direct culture tests performed by the American Type Cell Collection (Manassas, VA, USA).

Experimental design. The study procedures followed the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (30). This study (YAN31) was approved by the Institutional Animal Care and Use Committee of the Grand Forks Human Nutrition Research Center.

Three to four-week old WT or *Mcp1*$^{-/-}$ mice, after acclimation to the AIN93G diet for 1 week, were assigned to two groups (n=30-45 mice per group) and fed the AIN93G diet or HFD with deionized water for the duration of the study. Mice were weighed weekly. Three weeks following the initiation of the HFD, food intake (six mice per group) was recorded daily for 3 consecutive weeks. Body composition was determined using an Echo Whole Body Composition Analyzer (Model 100, Echo Medical Systems, Houston, TX, USA) 1 week prior to injection of LLC cells.

Eight weeks after the initiation of HFD, mice were subcutaneously injected with 2.5×10^5 viable LLC cells per mouse into the lower dorsal region. The resulting primary tumor was surgically removed 11 days later when it was approximately 1 cm in diameter. After surgery, mice were maintained on their respective diets for an additional 10 days. At termination, mice were euthanized by an intraperitoneal injection of a mixture of ketamine and xylazine. Lungs were harvested and fixed with Bouin's solution. The number of pulmonary metastases per mouse and the size (cross-sectional area and volume) of each metastasis were determined using a camera-equipped stereomicroscope and an ImagePro-Plus software (Media Cybernetics, Silver Spring, MD, USA) as described previously (31). Mice with birth defects, injection failure of LLC cells, or tumor recurrence after surgery were excluded from the study. Plasma and epididymal adipose tissue and were collected and stored at −80˚C for further analyses.

RNA isolation and real-time quantitative polymerase chain reaction (qPCR). Total RNA was isolated from frozen epididymal adipose tissue using QIAzol Lysis reagent with DNAse treatment following the manufacturer's protocol (RNeasy Lipid Tissue Mini Kit mRNA; Qiagen, Germantown, MD, USA). The quality and quantity of the RNA were analyzed by Nanodrop (Thermo Scientific, Wilmington, DE, USA). cDNA was synthesized using High Capacity cDNA reverse transcription Kit (Applied Biosystems, Waltham, MA, USA) following the manufacturer's protocol. Real-time qPCR of *Mcp1* was analyzed and normalized to that of *18s* rRNA using the TaqMan Assay of Demand primers on the ABI QuantStudio 12K-Flex Real-time PCR system (Applied Biosystems). The $2^{-\Delta\Delta CT}$ method was used to calculate the relative changes in gene expression (32).

Quantification of cytokines, angiogenic factors, and metabolic markers. Milliplex MAP mouse adipokine (MADKMAG-71K) and angiogenesis/growth factor (MAGPMAG-24K) magnetic bead panels (Millipore Sigma, Burlington, MA, USA) were used to quantify adipokines [leptin, MCP1, and plasminogen activator inhibitor-1 (PAI1)] and angiogenesis factors (VEGF, HGF, and angiopoietin-2) in plasma. Sandwich enzyme-linked immunosorbent assay kits were used to quantify insulin (Mercodia, Winston-Salem, NC, USA) in plasma, MCP1 (R&D Systems, Minneapolis, MS, USA) in adipose tissue, and adiponectin (R&D Systems) in both plasma and adipose tissue. The accuracy of the analysis was confirmed with controls provided in each kit; all samples analyzed were within the linear range of the assay.

Statistical analyses. The effects of diet (AIN93G or HFD), genotype (*Mcp1*$^{-/-}$ or WT), and their interaction were tested by using two-way analysis of variance (ANOVA). If a significant interaction between diet and genotype occurred, Tukey contrasts were performed to compare the four dietary groups. A mixed model ANOVA with 'mouse' as the random blocking factor and with diet, genotype, and their interaction as fixed effects was used to compare differences in cross-sectional area and volume of metastases among the four groups. All results are presented as means±standard error of the mean (SEM). Differences with a p-value of 0.05 or less were considered significant. All analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA).

Results

Regardless of genotype, mice fed the HFD were heavier than those fed the AIN93G diet; the difference was significant 2 weeks after the initiation of the HFD ($p<0.05$) and continued

Table I. *Composition of diets used in this study.*

Ingredient	AIN93G g/kg	High-fat g/kg
Corn starch	397.5	42.5
Casein	200	239.4
Dextrin	132	239.4
Sucrose	100	119.7
Soybean oil	70	239.4
Cellulose	50	59.8
AIN93 mineral mix	35	41.9
AIN93 vitamin mix	10	12
L-Cystine	3	3.6
Choline bitartrate	2.5	3
t-Butylhydroquinone	0.014	0.017
Total	1000	1000
Energy		
Protein	20%	20%
Fat	16%	45%
Carbohydrate	64%	35%
Analyzed gross energy, kcal/g[a]	4.3±0.1	5.2±0.1

[a]Values are means±SEM of three samples analyzed from each diet.

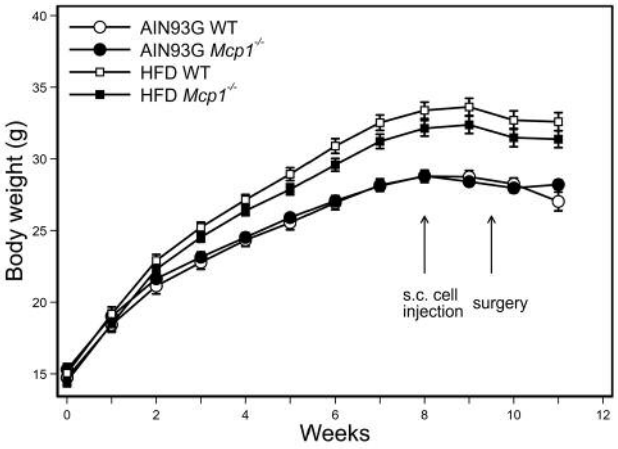

Figure 1. *Body weight of wild-type (WT) and adipose monocyte chemotactic protein-1 knockdown ($Mcp1^{-/-}$) mice fed the AIN93G or the high-fat diet (HFD). Mice fed the HFD were heavier than those fed the AIN93G diet; the difference was significant 2 weeks after the initiation of the HFD ($p<0.05$) and continued throughout the study. Adipose MCP1 deficiency did not affect body weight when comparing adipose-$Mcp1^{-/-}$ mice to WT mice on the same diet. Values are means±SEM (n=30-45 per group).*

throughout the study (Figure 1). Adipose MCP1 deficiency did not affect body weight when comparing $Mcp1^{-/-}$ mice to WT mice fed the same diet (Figure 1).

The percentage body fat mass was 37% higher and the percentage body lean mass was 6% lower in the HFD-fed than in the AIN93G-fed mice, regardless of genotype (Figure 2A and B). The lean mass was slightly higher in the HFD-fed than in the AIN93G-fed mice (Figure 2C). The differences in the percentage fat mass, the percentage lean mass, and the lean mass between $Mcp1^{-/-}$ and WT mice were not significant, regardless of diet (Figures 2A-C). There were no significant differences in energy intake among the four groups regardless of diet and genotype (Figure 2D).

The expression of *Mcp1* mRNA in adipose tissue was 85% higher in mice fed the HFD than in mice fed the AIN93G diet regardless of genotype, and it was 37% lower in $Mcp1^{-/-}$ mice than in WT mice regardless of diet (Figure 3A). Adipose concentration of MCP1 in HFD-fed WT mice was 87% higher than in their AIN93G-fed counterparts, while that in HFD-fed $Mcp1^{-/-}$ mice was 51% lower than in WT mice were fed the same diet (Figure 3B). Similarly, the plasma concentration of MCP1 in HFD-fed WT mice was 35% higher than in their AIN93G-fed counterparts, while that in HFD-fed $Mcp1^{-/-}$ mice was 25% lower than in their WT counterparts (Figure 3C).

All mice subcutaneously injected with LLC cells developed a primary tumor at the injection site and metastases in the lungs. There was no significant difference in the number of lung metastases between $Mcp1^{-/-}$ and WT mice fed the AIN93G diet (Figure 4A). Compared to the AIN93G diet, the HFD increased the number of metastases by 110% in WT mice (Figure 4A). The number of metastases was 27% lower in $Mcp1^{-/-}$ mice fed the HFD than in WT mice fed the same diet (Figure 4A). Regardless of genotype, the HFD increased metastatic cross-sectional area by 16% and volume by 19% compared to the AIN93G diet (Figure 4B and C). Regardless of diet, adipose MCP1 deficiency reduced metastatic volume by 14% but not cross-sectional area compared to WT mice (Figure 4B and C).

There were no differences in plasma concentrations of insulin and resistin between WT and $Mcp1^{-/-}$ mice fed the AIN93G diet (Table II). Concentrations of insulin and resistin were 78% and 45%, respectively, higher in HFD-fed WT mice than in their AIN93G-fed counterparts (Table II). Concentrations of insulin and resistin were 47% and 26%, respectively, lower in HFD-fed $Mcp1^{-/-}$ mice than in their WT counterparts (Table II).

There were no significant differences in plasma concentrations of leptin and PAI1 between WT and $Mcp1^{-/-}$ mice fed the AIN93G diet (Table II). Concentrations of leptin and PAI1 were 310% and 60%, respectively, higher in HFD-fed WT mice than in AIN93G-fed WT mice (Table II). Concentrations of leptin and PAI1 were 67% and 51%, respectively, lower in $Mcp1^{-/-}$ mice fed the HFD than in WT fed the HFD (Table II).

Plasma concentration of adiponectin was 21% lower in mice fed the HFD than in mice fed the AIN93G diet, regardless of genotype (Table II). Plasma adiponectin was 23% lower in $Mcp1^{-/-}$ mice than in WT mice, regardless of

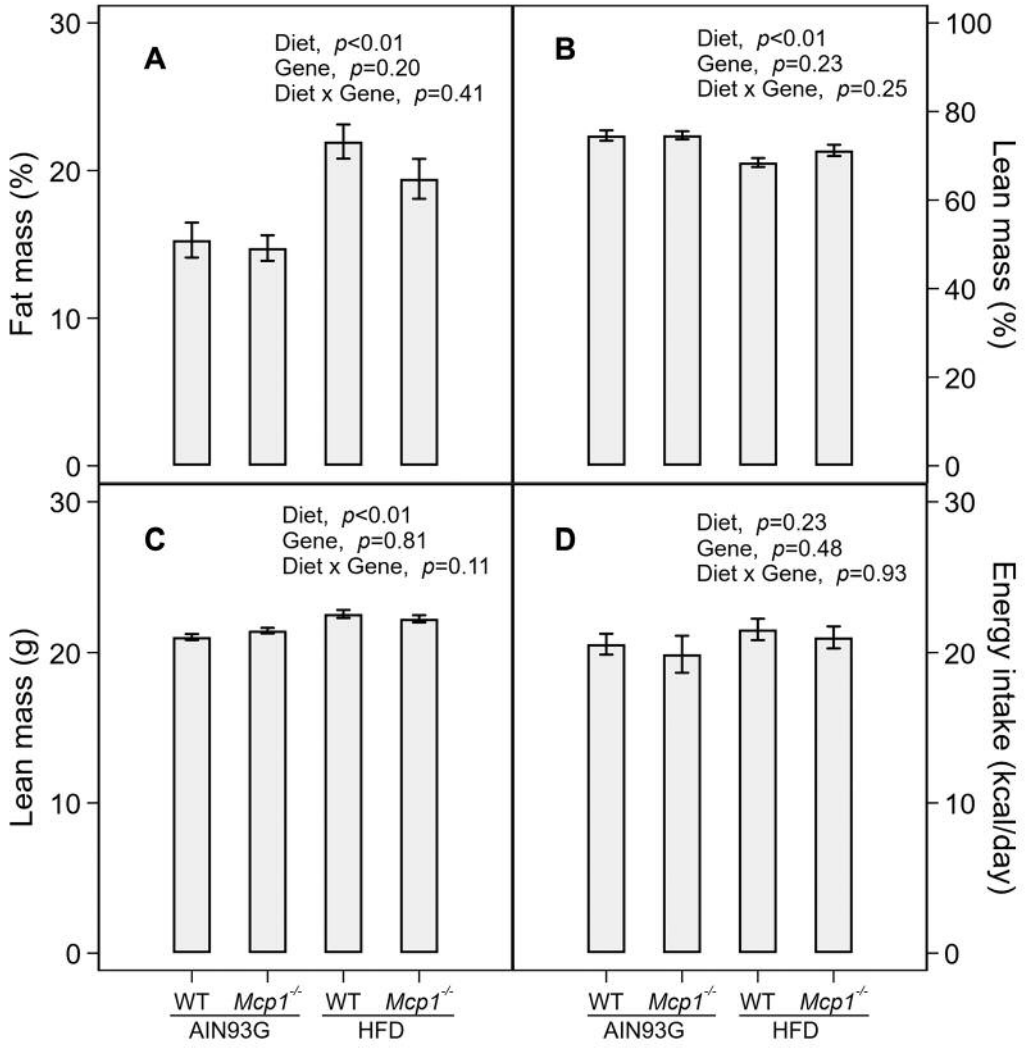

Figure 2. *The percentage body fat mass (A), the percentage body lean mass (B), the lean mass (C), and energy intake (D) of wild-type (WT) and adipose monocyte chemotactic protein-1 knockdown (Mcp1$^{-/-}$) mice fed the AIN93G or the high-fat diet (HFD). Values are means±SEM (n=30-45 per group for A, B, C; n=6 per group for D).*

diet (Table II). There was no difference in adipose concentration of adiponectin between the groups on the HFD and AIN93G diet, regardless of genotype; adipose adiponectin was 15% lower in *Mcp1*$^{-/-}$ mice than in WT mice, regardless of diet (Table II).

There were no differences in plasma concentrations of VEGF and HGF between *Mcp1*$^{-/-}$ and WT mice fed the AIN93G diet (Table II). The concentration of angiopoietin-2 was 38% lower in *Mcp1*$^{-/-}$ mice than in WT mice fed the AIN93G diet (Table II). Concentrations of VEGF, HGF, and angiopoietin-2 were 63%, 100%, and 41%, respectively, higher in HFD-fed WT mice than in AIN93G-fed WT mice, while they were 28%, 70%, and 68%, respectively, lower in HFD-fed *Mcp1*$^{-/-}$ mice than in HFD-fed WT mice (Table II).

Discussion

Consistent with our previous reports (17, 25, 33), consumption of an obesogenic HFD enhanced spontaneous metastasis of LLC as evidenced by increases in the number and size of metastases formed in the lungs. In the present study, the number of lung metastases from adipose *Mcp1*$^{-/-}$ mice fed the HFD was fewer than that from their WT counterparts, and the metastatic volume from *Mcp1*$^{-/-}$ mice was smaller than that from WT mice. Concentrations of MCP1 in adipose tissue and plasma from *Mcp1*$^{-/-}$ mice were lower than those from WT mice. These findings indicate that knockdown of MCP1 from adipose tissue may be responsible for the observed attenuation in HFD-enhanced metastasis and

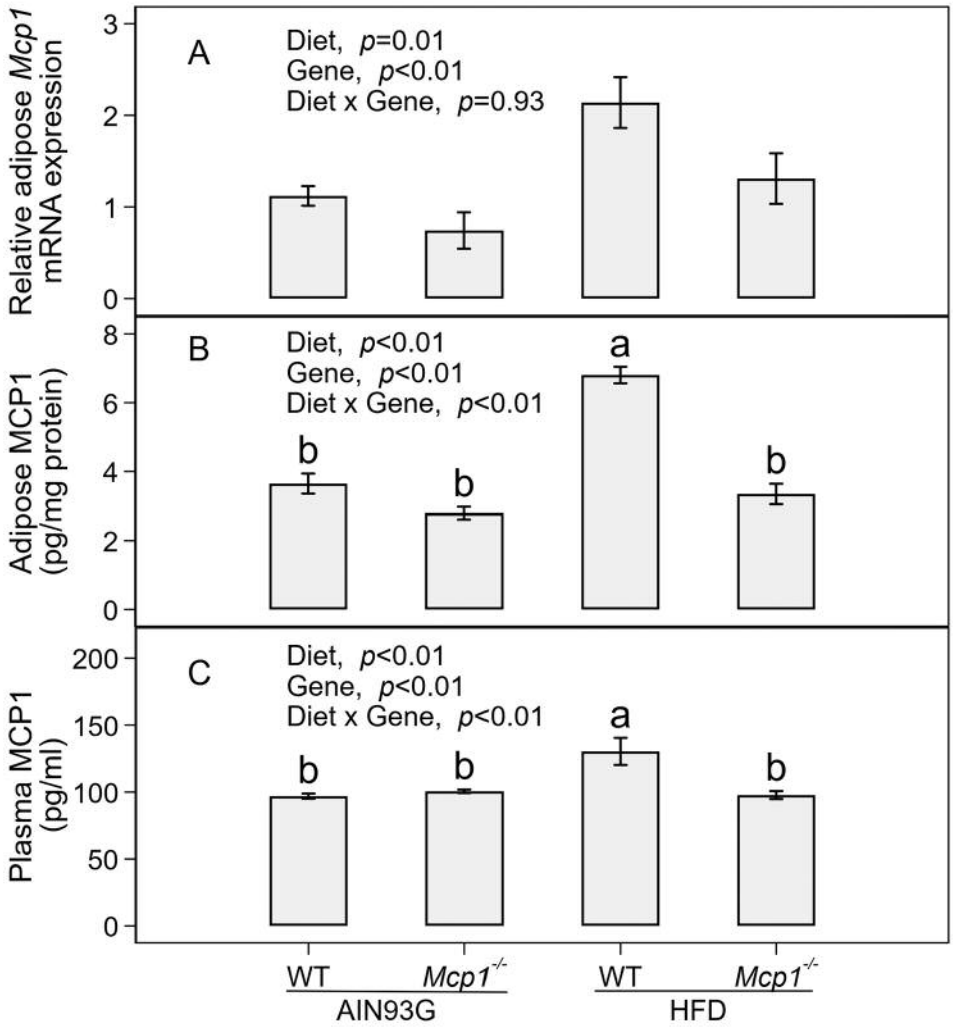

Figure 3. *Expression of Mcp1 mRNA in adipose tissue (A) and concentrations of MCP1 in adipose tissue (B) and plasma (C) from wild-type (WT) and adipose monocyte chemotactic protein-1 knockdown ($Mcp1^{-/-}$) mice fed the AIN93G or the high-fat diet (HFD). Values (means±SEM) with different letters are different at p<0.05 (n=7-8 per group for A; n=15-16 per group for B and C).*

support our hypothesis that adipose-produced MCP1 contributes to malignant spread.

Mice with adipose MCP1 deficiency exhibited lower plasma concentrations of PAI1, leptin, and resistin, particularly those fed the HFD. PAI1 (34, 35), leptin (36, 37), and resistin (38, 39) are proinflammatory cytokines with demonstrated cancer-promoting potential. Depletion of PAI1 inhibited spontaneous metastasis of LLC (33), knockout of leptin receptors mitigated mammary tumorigenesis mediated by mouse mammary tumor virus (40), and inhibition of resistin expression suppressed experimental metastasis of chondrosarcoma in mice (41). Adipocyte hypertrophy and hyperplasia is a hallmark of obesity, in which expression of MCP1 increases the recruitment of monocytes and macrophages to adipose tissue (42). Adipogenesis results in further elevation of proinflammatory adipokines and facilitation of obesogenesis (43, 44). Thus, it is likely that adipose MCP1 deficiency may result in an active anti-inflammatory process or a blockade of an inflammatory process that reduces monocyte and macrophage infiltration and production of inflammatory cytokines, including PAI1, leptin, and resistin. These decreases may be responsible, at least partly, for the observed attenuation in diet-enhanced metastasis.

Angiogenesis is important for both adipogenesis and tumorigenesis. Elevated expression of MCP1 is found in both obesity (42) and cancer (22, 45); this elevation is accompanied with increases in expression of angiogenic factors. For

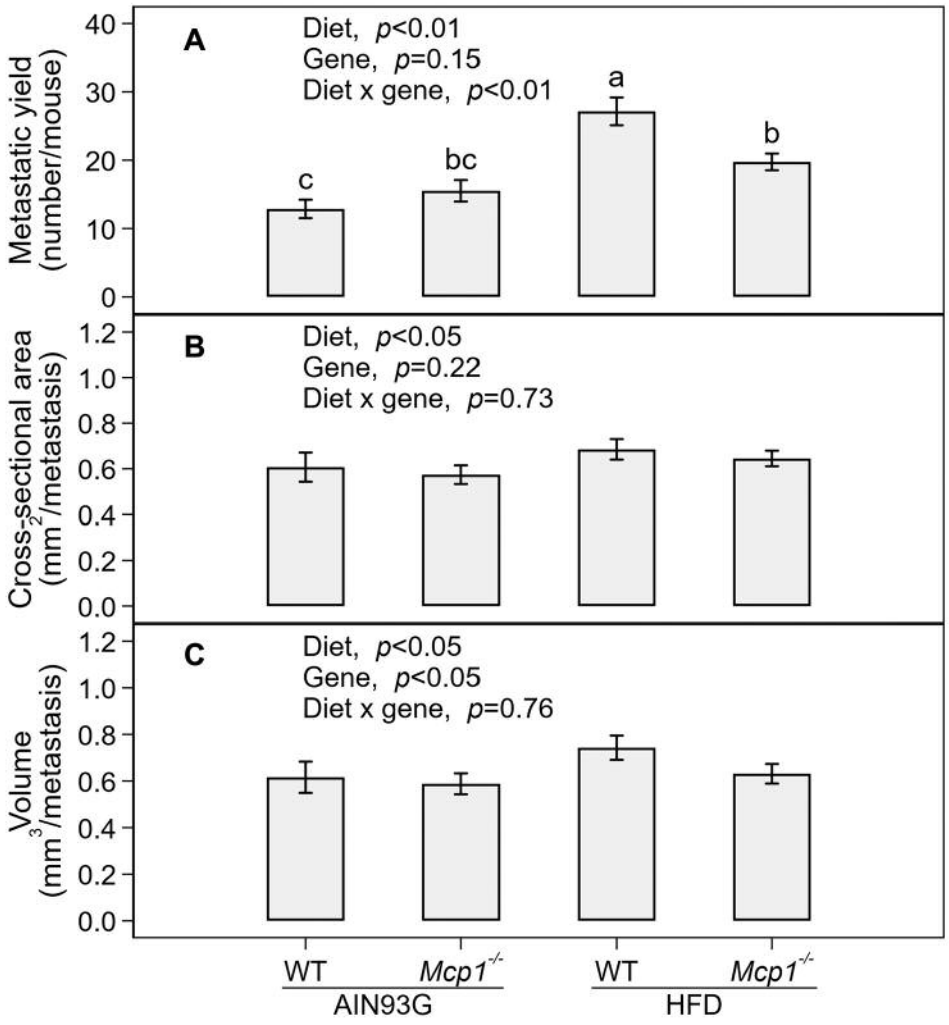

Figure 4. *The number (A), cross-sectional area (B), and volume (C) of lung metastases in wild-type (WT) and adipose monocyte chemotactic protein-1 knockdown ($Mcp1^{-/-}$) mice fed the AIN93G or the high-fat diet (HFD). Values (means±SEM) with different letters are different at p<0.05 (n=27-42 per group).*

example, elevated expression of both MCP1 and VEGF are found during macrophage infiltration and macrophage-mediated angiogenesis in human gastric carcinoma (45) and in primary breast cancer specimens from patients with early relapse (46). Incubation of macrophages in culture with MCP1 enhances HGF production (47). Elevated expression of MCP1 was found to correlate with the expression of HGF in leukemia (48) and ovarian cancer (49) and the expression of angiopoietin-2 in colorectal cancer metastasis to the liver (4). The knockdown of MCP1 in adipose tissue may interfere with the coordinated regulatory pathways between MCP1 and angiogenic factors during malignant progression. This may contribute to the observed attenuation in metastasis because adipose $Mcp1^{-/-}$ mice exhibited lower concentrations of VEGF, HGF, and angiopoietin-2 in plasma in the present study.

Lower insulin and resistin concentrations in $Mcp1^{-/-}$ mice fed the HFD indicate that adipose MCP1 deficiency may improve insulin sensitivity and metabolic homeostasis. This is further supported by the finding that $Mcp1^{-/-}$ mice exhibited a lower concentration of leptin in plasma. Resistin and leptin are positively correlated with insulin resistance in humans (50) and laboratory rodents (14, 15). Mice with lower plasma concentrations of insulin and leptin (16) or deficiency in resistin (13), induced by experimental means, exhibit improved energy metabolism (16), glucose tolerance, and insulin sensitivity (13). Furthermore, lower insulin concentration in the present study may also have contributed to the attenuation in malignant spread in $Mcp1^{-/-}$ mice, because insulin was found to be tumorigenic in a mouse model of mouse mammary tumor virus-polyomavirus middle T-antigen (51).

Table II. *Concentrations of insulin, adipokines, and angiogenic factors in plasma and concentrations of adiponectin in adipose tissue of wild-type (WT) and adipose monocyte chemotactic protein-1 knockdown (Mcp1$^{-/-}$) mice fed the AIN93G or the high-fat diet.*

	Diet						
	AIN93G		High-fat		*p*-Value		
Genotype	WT	*Mcp1*$^{-/-}$	WT	*Mcp1*$^{-/-}$	Diet	Gene	Interaction
Insulin, pg/ml	186.21±22.60^{b}	184.63±30.52^{b}	330.90±46.14^{a}	176.29±36.80^{b}	0.06	<0.05	<0.05
Resistin, ng/ml	1.10±0.08^{b}	1.11±0.08^{b}	1.60±0.10^{a}	1.18±0.12^{b}	<0.01	<0.05	<0.05
Leptin, ng/ml	0.67±0.13^{b}	0.38±0.07^{b}	2.75±0.66^{a}	0.90±0.26^{b}	<0.01	<0.01	<0.05
PAI1, ng/ml	1.16±0.11^{b}	0.92±0.11^{b}	1.85±0.20^{a}	0.91±0.06^{b}	<0.01	<0.01	<0.01
Adiponectin, μg/ml	8.15±0.57	6.82±0.75	7.06±0.45	4.84±0.90	<0.05	0.01	0.52
Adiponectin, μg/mg protein	0.90±0.07	0.73±0.03	0.85±0.06	0.77±0.04	0.90	0.01	0.29
VEGF, ng/ml	0.35±0.04^{b}	0.33±0.03^{b}	0.57±0.02^{a}	0.41±0.03^{b}	<0.01	<0.01	<0.05
HGF, ng/ml	0.48±0.08^{b}	0.43±0.08^{b}	0.96±0.18^{a}	0.29±0.03^{b}	0.13	<0.01	<0.01
Angiopoietin-2, ng/ml	4.80±0.55^{b}	2.99±0.53^{c}	6.76±0.43^{a}	2.18±0.20^{c}	0.20	<0.01	<0.01

PAI1: Plasminogen activator inhibitor-1; VEGF: vascular endothelial growth factor; HGF: hepatic growth factor. Values (means±SEM) with different letters are different at $p<0.05$ (n=14-16 per group).

The present study supports our previous report that whole-body *Mcp1* knockout reduced HFD-enhanced spontaneous metastasis of LLC in mice (25). It further demonstrates the importance of adipose-produced MCP1 in malignant spread. A difference between these two studies is that the elevation of proinflammatory and angiogenic markers in whole-body *Mcp1* knockout mice (25), whereas these markers were lower in adipose in *Mcp1*$^{-/-}$ mice. These findings show the dependence of LLC on MCP1 and the existence of a compensatory mechanism that provides support for malignant aggression in the absence of MCP1. Furthermore, the observed lower concentrations of proinflammatory and angiogenic markers caused by *Mcp1* knockdown in adipose tissue show that MCP1 participates in the regulation of inflammation and angiogenesis during adipogenesis and tumorigenesis.

It was anticipated that concentrations of adiponectin in both plasma and adipose tissue were low in adipose *Mcp1*$^{-/-}$ mice. Adiponectin is an adipocyte specifically secreted protein with roles in glucose and lipid homeostasis (52). Blood levels of adiponectin are elevated with improvement in insulin sensitivity (52). In this study, adipose-specific *Mcp1* knockdown was achieved with Cre-recombinase driven by the adiponectin promoter (*Mcp1*$^{fl/fl}$/*Adipoq-Cre*$^{+}$). Addition of Cre-recombinase to the Adipoq locus leads to transcription and translation of that locus to be disabled, because of replacement of 222 bp of adiponectin gene (53). This may explain the reduced expression of adiponectin in adipose *Mcp1*$^{-/-}$ mice in the present study.

A potential limitation of this study is the partial depletion of MCP1 from adipose tissue, which makes this model unable to examine the effect of a complete adipose *Mcp1* knockout on metastasis. Nevertheless, we demonstrated the importance of adipose-produced MCP1 in metastasis with this model.

In summary, the present study showed that adipose *Mcp1* knockdown attenuated HFD-enhanced spontaneous metastasis of LLC. This attenuation was accompanied with lower concentrations of proinflammatory and angiogenic markers in plasma, which indicates that adipose MCP1 deficiency may down-regulate inflammation and angiogenesis during malignant aggression. We conclude that adipose-produced MCP1 contributes to HFD-enhanced metastasis.

Conflicts of Interest

The Authors have declared that no competing interests exist in regard to this study.

Authors' Contributions

LY and SS contributed to the conception and design of the study, data interpretation, and writing of the manuscript. Both Authors contributed to review and revision of the manuscript and agreed to be accountable for the content of the work.

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