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Regulation of Adipose Tissue Inflammation and Insulin Resistance by MAPK Phosphatase 5*

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Background: MKP5 regulates inflammation in innate immunity.
Results: MKP5 knock-out mice developed insulin resistance and glucose intolerance spontaneously, associated with increased inflammatory macrophage infiltration in visceral adipose tissue.
Conclusion: MKP5 critically controls adipose tissue inflammation and insulin sensitivity.
Significance: Understanding the role of MKP5 in metabolism will shed new light on the pathogenesis and treatment of metabolic disorders.

Obesity and metabolic disorders such as insulin resistance and type 2 diabetes have become a major threat to public health globally. The mechanisms that lead to insulin resistance in type 2 diabetes have not been well understood. In this study, we show that mice deficient in MAPK phosphatase 5 (MKP5) develop insulin resistance spontaneously at an early stage of life and glucose intolerance at a later age. Increased macrophage infiltration in white adipose tissue of young MKP5-deficient mice correlates with the development of insulin resistance. Glucose intolerance in MKP5-deficient mice is accompanied by significantly increased visceral adipose weight, reduced AKT activation, enhanced p38 activity, and increased inflammation in visceral adipose tissue when compared with wild-type (WT) mice. Deficiency of MKP5 resulted in increased inflammatory activation in macrophages. These findings thus demonstrate that MKP5 critically controls inflammation in white adipose tissue and the development of metabolic disorders.

Type 2 diabetes stems from the failure of the body in responding appropriately to insulin. Insulin resistance has been closely associated with obesity and inflammation (1, 2). Adipose tissue dysfunction is a primary defect in obesity and obesity-associated metabolic diseases (3). By coordinating through a number of local and systemic effectors, adipose tissue plays a major role in metabolic homeostasis. Inflammation is a key feature of obesity and type 2 diabetes (4). Within adipose tissue microenvironment, adipocytes secrete adipokines, including leptin, adiponetin, and resistin to regulate systemic lipid and glucose metabolism (5, 6). However, immune cells such as macrophages and T cells that infiltrated into adipose tissue in obesity are the major source of inflammatory cytokines, including TNFα, IL-β, and IL-6 (7). These inflammatory mediators regulate adipocyte function and insulin sensitivity directly and indirectly.

Intracellular signaling pathways that regulate inflammation, such as MAPK pathway, have been shown to regulate insulin sensitivity, obesity, and the pathogenesis of diabetes (8–11). For instance, JNK[5] activation in insulin responsive tissues, including skeletal muscle, the liver, and adipose tissue, is abnormally elevated in obesity (9). Mice deficient in JNK1 exhibited enhanced insulin signaling and were protected from obesity-

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[5] The abbreviations used are: JNK, c-Jun N-terminal kinase; MKP, MAPK phosphatase; BMDM, bone-marrow-derived macrophage; ATM, adipose tissue macrophage; ERK, extracellular signal-regulated kinase; KO, knock-out; SVC, stromal vascular cell; qPCR, quantitative RT-PCR; vWAT, visceral WAT; sWAT, subcutaneous WAT.

induced insulin resistance. Ser-307 of IRS-1, an inhibitory phosphorylation site, is a target of JNK. TNFα inhibits the signaling capacity of the insulin receptor by inducing Ser-307 phosphorylation through JNK (9, 12). Mice deficient in ERK1 are resistant to diet-induced obesity and insulin resistance due to impaired adipogenesis (13). Therefore, tight control of MAPK pathways is essential for preventing the development of metabolic disorders.

MAPK phosphatases (MKPs) are key negative regulators of MAPKs (14, 15). We previously found that one MKP protein family member, MKP5, inhibits proinflammatory cytokine production in both innate and adaptive immune responses (16). MKP5 is abundantly expressed in insulin responsive tissues and organs such as skeletal muscle and liver in both human and mouse (17, 18). The regulation of inflammation by MKP5 and its expression in insulin responsive tissues and organs suggest possible involvement of this protein in metabolic regulation. In the present study, we investigated the function of MKP5 in adipose tissue inflammation and insulin sensitivity.

Experimental Procedures

Animal Experiments—Mice were housed in specific pathogen-free animal facilities at the University of Texas MD Anderson Cancer Center and the National University of Singapore. Animal experiments were performed in accordance with protocols reviewed and approved by the University of Texas MD Anderson Cancer Center Institutional Animal Care and Utilization Committee and the National University of Singapore Institutional Animal Care and Use Committee. The MKP5-deficient mice generated previously (16) were crossed with C57BL/6 mice for 10 generations. Insulin tolerance test was performed by injecting 0.7 units of insulin/kg body weight into the peritoneum. Blood glucose was measured before and after injection using an Ascensia Elite XL glucose meter and test strips (Bayer Healthcare). Glucose tolerance test was performed on overnight-fasted mice similarly by injecting 2 g of D-glucose/kg body weight. Hyperinsulinemic-euglycemic clamp studies were performed at the UMASS Mouse Phenotyping Center as described previously (19, 20). Briefly, after an overnight fast, a 2-h hyperinsulinemic-euglycemic clamp was conducted in conscious mice with a primed and continuous infusion of human insulin (150 milliunits/kg body weight priming followed by 2.5 milliunits/kg/min; Humulin, Eli Lilly), and 20% glucose was infused at variable rates to maintain euglycemia. Whole body glucose turnover was assessed with a continuous infusion of [3-^{3}H]glucose. Mice were anesthetized at the end of clamps.

Isolation of Adipose Tissue Stromal Vascular Cells—Isolation of stromal vascular cells (SVCs) was performed as described previously (21). Briefly, perigonadal fat pads were taken from mice immediately after CO_2 asphyxiation, minced, and digested in LPS-depleted collagenase mixture (Roche Applied Science) plus DNase I (Sigma-Aldrich) at 37 °C for 45–60 min. Samples were passed through a sterile 250-μm nylon mesh, and the suspension was centrifuged at 1,000 $\times$ *g* for 10 min. The pelleted cells were collected as the SVCs.

Cells and Cell Culture—Bone marrow cells were flushed out with PBS from the femur of the mice. Red blood cells were lysed with ACK lysis buffer. The cells were then cultured in RPMI 1640 medium supplemented with 10% FBS, 1.0 units/ml penicillin, 1 μg/ml streptomycin, and 20 ng/ml M-CSF (Peprotech) for bone-marrow-derived macrophages (BMDMs). After 7 days of differentiation, cells were harvested for analysis. Pre-adipocytes isolated from visceral white adipose tissue (WAT) were differentiated into mature adipocytes in Dulbecco's modified Eagle's medium (DMEM) supplied with fetal calf serum, insulin (Sigma), L-glutamine, and sodium ascorbate (Sigma) for 7 days. Co-culture of adipocytes and macrophages was performed in a contact system where differentiated adipocytes were cultured in a six-well plate, and 10^5 BMDMs were plated onto adipocytes. Cells were harvested for RNA extraction and gene expression analysis. Culture supernatants were harvested to determine cytokine secretion. As a control, adipocytes and macrophages, the numbers of which were equal to those used in the co-culture were cultured separately and mixed together for gene expression.

Expression Analysis—Total RNA was extracted from the liver, adipose tissue, and adipose tissue macrophages (ATMs) using TRIzol reagent (Invitrogen). cDNA was prepared using Superscript reverse transcriptase and oligo(dT) primers (Invitrogen). SYBR Green-based quantitative RT-PCR (qPCR) was performed using β-actin as a housekeeping gene control. For Western blot analysis, tissues were prepared in Triton lysis buffer containing protease and phosphatase inhibitors. Protein was fractionated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-phospho-p38 (Cell Signaling) or anti-p38 (Santa Cruz Biotechnology) antibodies.

Histological Analysis—Tissues were fixed overnight in Bouin's solution (Sigma) and washed thoroughly in running water before dehydration, and tissues were embedded in paraffin and sectioned for H&E staining. Paraffin sections of visceral WAT were also stained with anti-mouse F4/80 antibody (eBioscience).

Statistical Analysis—Statistical analysis was calculated with an unpaired Mann-Whitney test and STATISTICA software (StateSoft, Inc., Tulsa, OK). We used individual glucose values in insulin resistance test and glucose tolerance test assays at each time point for statistical analysis. p values of 0.05 or less were considered significant.

Results

MKP5 Deficiency in Mice Resulted in Increased Adiposity—MKP5 has been shown to be abundantly expressed in insulin responsive tissues/organs such as skeletal muscle and the liver in both human and mouse (17, 18). We further examined its expression in WAT and found that it is highly expressed in WAT in mouse and its expression is greatly up-regulated in WAT from older mice (Fig. 1*A*). To investigate the role of MKP5 in obesity, we compared the body weight of male and female WT and MKP5 knock-out (KO) mice at different ages. At the age of three months, there was no significant difference in body weight between WT and MKP5 KO mice in both males and females, respectively. Significantly increased body weight was observed in MKP5 KO male mice at the age of 5 months compared with age-matched WT mice (Fig. 1*B*). Increased body weight was also observed in MKP5 KO female mice at the

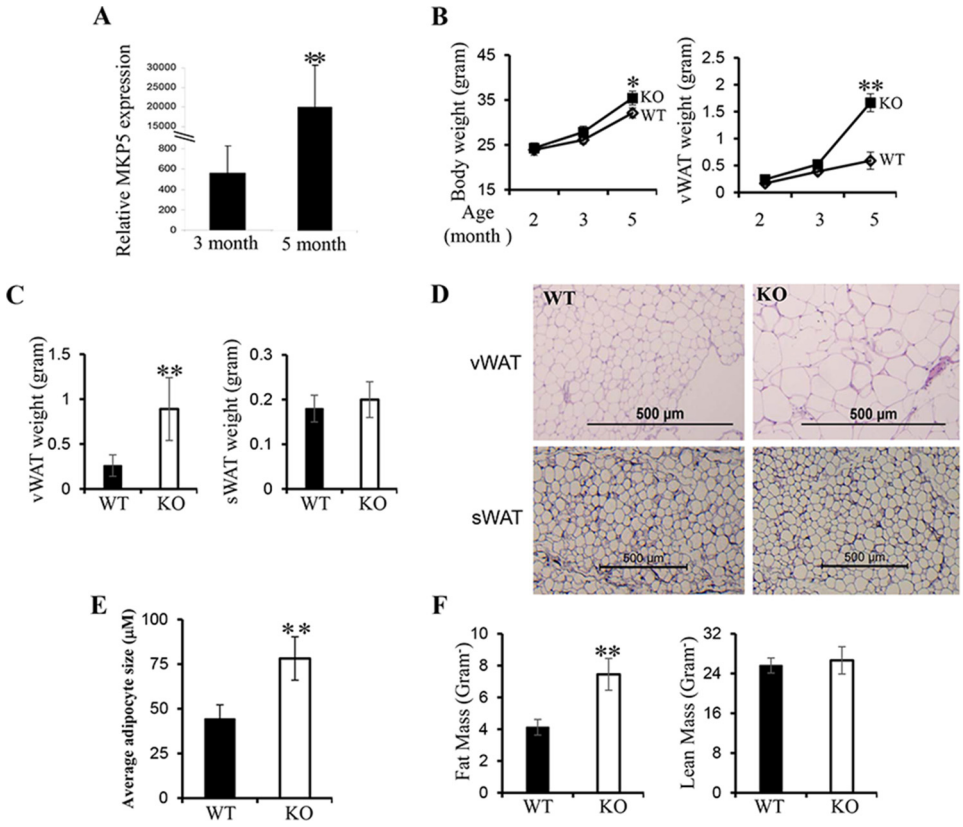

FIGURE 1. **Deficiency of MKP5 in mice resulted in the development of visceral obesity.** *A*, MKP5 mRNA expression in perigonadal adipose tissue (visceral WAT) from 3- and 5-month-old C57BL/6 (WT) mice was determined by SYBR green-based qPCR. *B*, body weight and the weight of visceral WAT (vWAT) of age-matched male WT and MKP5 KO mice were determined. *C*, the weight of vWAT and subcutaneous WAT (sWAT) from age-matched female mice was measured. *D* and *E*, vWAT and sWAT were taken from 5-month old WT and MKP5 KO female mice ($n = 4–5$), and H&E staining was performed. The size of adipocytes was measured digitally with ImageJ software. *F*, fat mass and lean mass of 5-month-old WT and MKP5 KO male mice was determined by NMR. *, $p < 0.05$; **, $p < 0.01$.

age of 5 months compared with WT mice, although the difference did not reach statistical significance (data not shown). However, both female and male mice at the age of 5 months had larger perigonadal fat pads compared with WT mice (Fig. 1, *B* and *C*). Consistent with increased weights, histological analysis of perigonadal WAT from 5-month-old KO mice exhibited increased size of adipocytes compared with those from age-matched WT mice (Fig. 1, *D* and *E*), whereas the size of adipocytes is comparable in subcutaneous WAT between WT and KO mice (Fig. 1*D*). We refer to the perigonadal fat tissue as visceral WAT, as it is considered as "pure" white adipose tissue and is the major component of visceral adipose tissue (22, 23). The weights of various organs, including liver, pancreas, spleen, and brown adipose tissues were comparable between WT and MKP5 KO mice (data not shown). Nuclear magnetic resonance (NMR) spectroscopy examination of the body composition of WT and KO mice at the age of 3 months (data not shown) and 5 months demonstrated that the average fat mass of the KO mice was significantly higher than that of WT mice, whereas the lean mass of WT and KO mice was comparable (Fig. 1*F*). These results demonstrated that deficiency of MKP5 resulted in increased adiposity in mice, especially in visceral WAT.

MKP5-deficient Mice Develop Insulin Resistance in an Early Stage of Life—Excessive accumulation of adipose tissue, particularly visceral adipose tissue, is associated with the development of metabolic disorders such as insulin resistance. Therefore, we compared insulin sensitivity between WT and MKP5 KO mice. We found that both male and female KO mice had significantly decreased insulin sensitivity starting at age of 3 months compared with WT mice (Fig. 2*A*). At this stage, MKP5 KO mice had normal responses to glucose injection when assessed in glucose tolerance tests (Fig. 2*A*). To validate the role of MKP5 in insulin sensitivity, we performed hyperinsulinemic-euglycemic clamps in both WT and MKP5 KO mice at various ages. We found that at the age of 2 months, some of the MKP5 KO mice began to be insulin resistant as determined by glucose infusion rates compared with WT mice (data not shown). At the age of 3 months or above, as shown in Fig. 2*B*, MKP5 KO mice had significant reductions of glucose infusion rates and whole body glucose turnover compared with WT mice. Together, these results demonstrated that the deficiency of MKP5 resulted in impaired insulin sensitivity.

Obesity and insulin resistance is associated with a low grade inflammation in WAT (2). Therefore, we examined proinflammatory mediator expression in the WAT of both WT and MKP5 KO mice. The expression of IL-1β and TNFα was increased in the WAT of KO mice at the age of 2–3 months but was not statistically significant (Fig. 2*C*). Interestingly, the expression of monocyte chemoattractant protein-1 (MCP-1 or CCL2), which plays a critical role in the recruitment of macrophages and contributes to the development of insulin resistance (24), was significantly increased in MKP5 KO WAT than that in

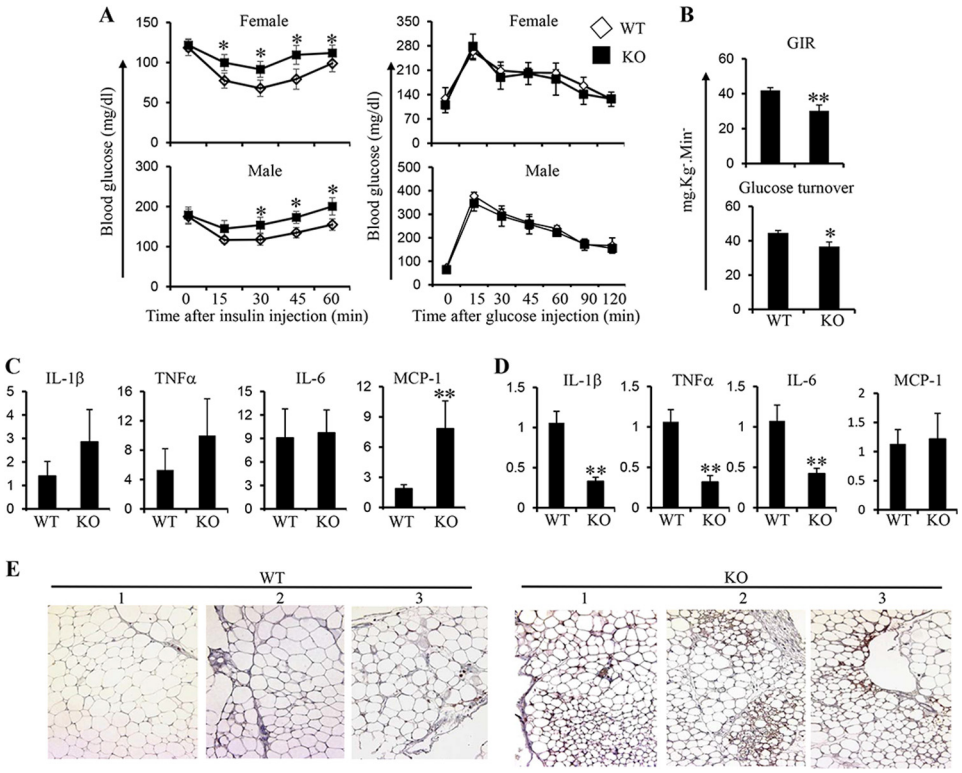

FIGURE 2. **MKP5 KO mice develop insulin resistance at an early stage of life.** *A*, insulin tolerance tests were performed in sex-matched 3-month old WT ($n = 10$) and MKP5 KO mice ($n = 9$) after injection of 0.7 units/kg human insulin (Sigma). Glucose tolerance tests were performed on sex-matched WT ($n = 10$) and MKP5 KO mice ($n = 9$) at the same age after they were fasted overnight by giving 2g/kg of D-glucose intraperitoneal blood glucose levels at the indicated time were measured with an Ascensia Elite XL glucose meter and test strips. The data shown are representative of six independent experiments with similar results. *B*, hyperinsulinemic-euglycemic clamps were performed on WT ($n = 8$) and MKP5 KO ($n = 5$) mice at the age of 3 and 5 months. Average glucose infusion rate (*GIR*) and whole body glucose turnover were determined. *C* and *D*, vWAT (*C*) and sWAT (*D*) were taken from 3-month-old WT and MKP5 KO female mice ($n = 4–5$) after fasting overnight. Indicated cytokine gene expression was determined by qPCR. *E*, F4/80 positive macrophages in vWAT of 3-month old WT and KO were determined by immunohistochemistry. The data shown are representative of two to three independent experiments with similar results. Data are presented as mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

WT WAT (Fig. 2*C*). However, reduced expression of inflammatory cytokines, including IL-1β, TNFα, and IL-6 was detected in subcutaneous WAT from KO mice compared with that from WT mice, whereas the expression of MCP-1 between WT and KO subcutaneous WAT is comparable (Fig. 2*D*). To address whether increased MCP-1 expression leads to increased macrophage recruitment, visceral WAT from WT and MKP5 KO mice at the age of 3 months was taken for histological analysis. We found dramatically increased macrophage infiltration in WAT from KO mice compared with those from WT mice (Fig. 2*E*).

MKP5-deficient Mice Develop Glucose Intolerance in a Late Stage of Life—We continuously monitored the metabolic status of MKP5 KO mice and found that at the age of 5 months, both male and female KO animals developed glucose intolerance (Fig. 3*A*) in addition to insulin resistance. At the same time, significantly increased fasting serum insulin concentrations were detected in MKP5 KO mice compared with WT mice (Fig. 3*B*). Interestingly, we detected increased serum concentration of c-peptide in MKP5 KO mice at the age of 3 months (Fig. 3*C*), indicating that compensation of insulin resistance by more insulin production already occurred at this stage. In line with the glucose intolerance and hyperinsulinemia at the age of 5 months, MKP5 KO mice have significantly increased fasting blood glucose levels compared with WT mice (Fig. 3*D*). AKT phosphorylation in both visceral and subcutaneous WAT from WT and KO mice at various ages was examined. We detected decreased AKT phosphorylation in visceral WAT, but not subcutaneous WAT, from 5-month-old MKP5 KO mice compared with that in WT WAT (Fig. 3*E*).

Obesity, inflammation, and insulin resistance are linked closely. We found increased expression of proinflammatory cytokines, including IL-1β, IL-6, TNFα, and MCP-1 in visceral WAT from 5-month-old KO mice compared with those from age-matched WT mice (Fig. 4*A*), whereas the expression of these genes in subcutaneous WAT was comparable (Fig. 4*B*). Moreover, sera from MKP5 KO mice contained significantly increased levels of IL-6 and TNFα than those from WT mice (Fig. 4*C*). Together, these results demonstrated that the development of obesity and glucose intolerance of MKP5 KO mice is associated with an inflammatory status of the mice.

We previously showed that MKP5-deficient immune cells had increased JNK activity (16). When we analyzed MAPK activation in insulin responsive tissues, we found that p38 activation was constitutively elevated in WAT of MKP5 KO mice (Fig. 4*D*), whereas no significant change of p38 activation was observed in the liver from WT and KO mice (data not shown). Similar ERK activation was detected in WT and MKP5 KO WAT and skeletal muscle (data not shown), and no JNK activation was found in these tissues. These results suggest that

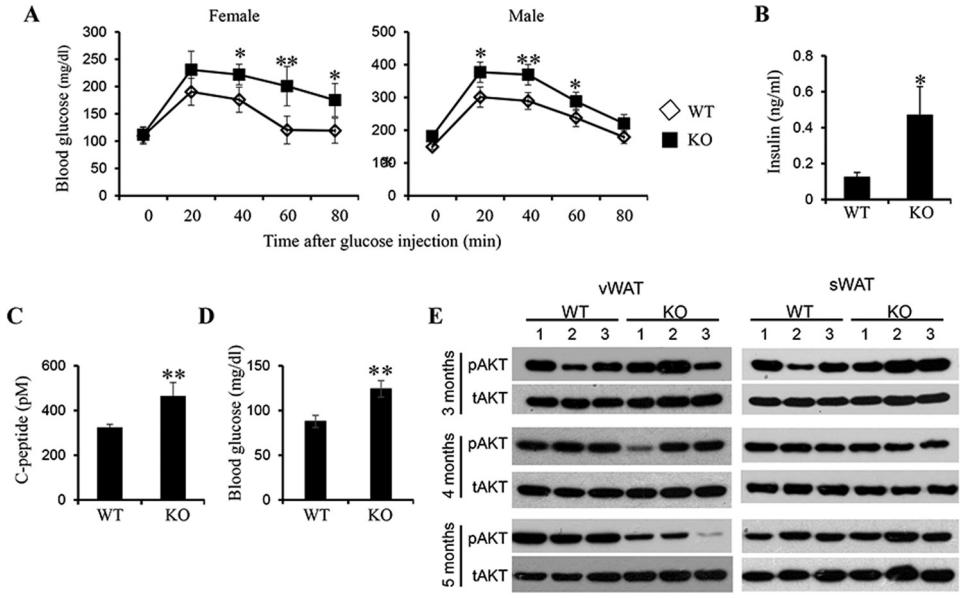

FIGURE 3. **MKP5 KO mice develop glucose intolerance and hyperinsulinemia at 5 months of age.** *A*, glucose tolerance tests were performed in male WT ($n = 10$) and MKP5 KO mice ($n = 8$) at the age of 5 months. *B*, fasting serum levels of insulin were determined by ELISA. *C*, fasting serum c-peptide concentrations in WT and MKP5 KO mice at the age of three months were determined by c-peptide ELISA kit (Salem, NH). *D*, fasting blood glucose levels of 5-month-old WT ($n = 5$) and KO mice ($n = 5$) were determined. *E*, AKT phosphorylation in vWAT and sWAT from 3-, 4-, or 5-month-old WT and MKP5 KO mice was examined by Western blot analysis. The data shown are representative of three independent experiments with similar results. Data are presented as mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

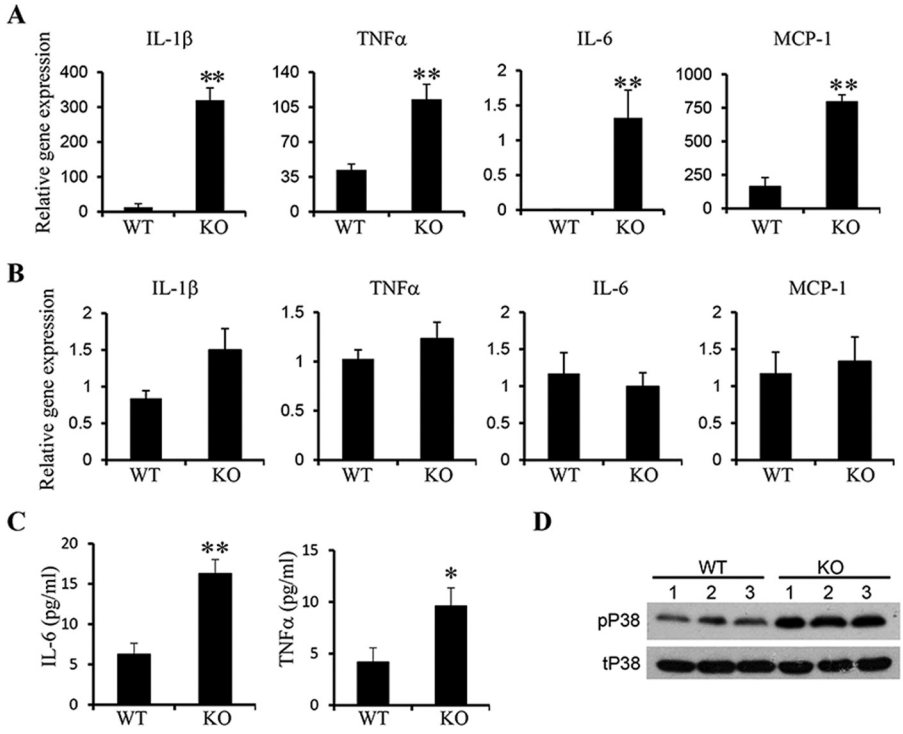

FIGURE 4. **Enhanced adipose tissue inflammation in MKP5 KO mice.** *A* and *B*, vWAT (*A*) and sWAT (*B*) were taken from 5-month-old WT and MKP5 KO female mice ($n = 4–5$). Cytokine gene expression was determined by qPCR. *C*, serum cytokines from 5-month-old WT and MKP5 KO female mice ($n = 4–5$) fasted overnight were determined by ELISA. *D*, p38 activity in vWAT of 4-month-old WT and MKP5 KO mice after fasting overnight was determined by Western blot analysis. The data shown are representative of two to three independent experiments with similar results. *, $p < 0.05$; **, $p < 0.01$.

MKP5 regulates different MAPK activation in a tissue- and cell-specific manner.

MKP5 Deficiency in Hematopoietic Cells Results in Insulin Resistance and Glucose Intolerance—To determine the contribution of hematopoietic and non-hematopoietic compartments of MKP5 KO mice to their development of metabolic disorders, we performed mixed bone marrow studies and generated mice lacking MKP5 in either hematopoietic cell or non-hematopoietic cell (radiation-resistant) compartments. Eight weeks after bone marrow transplantation, white blood cells displayed the donor genotype (Fig. 5*A*), indicating that the reconstitution is complete. Insulin tolerance tests revealed that only

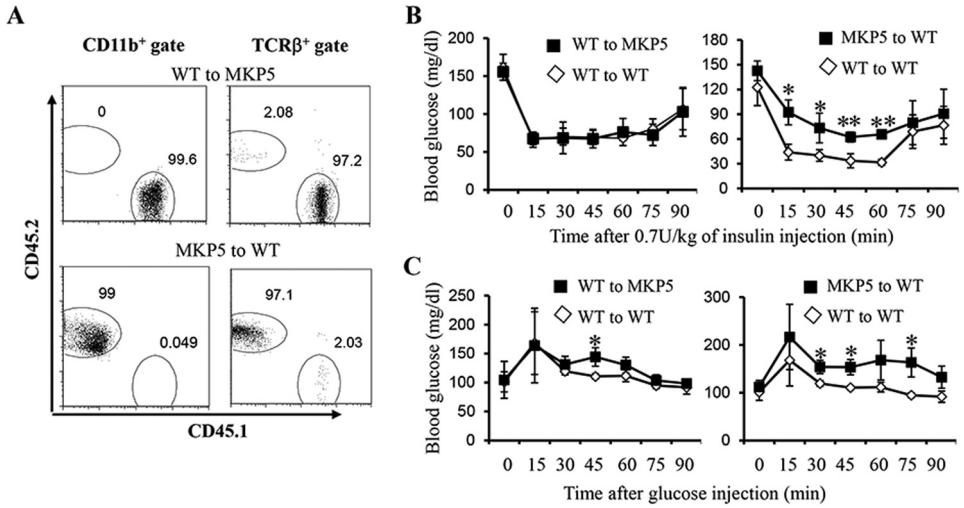

FIGURE 5. **MKP5 deficiency in hematopoietic cells caused metabolic disorders.** Six-week-old mice were lethally irradiated and were transferred with 5×10^6 bone marrow cells from donor mice. *A*, total white blood cells from various chimeras (WT to MKP5, B6SJ bone marrow (BM) cells transferred to MKP5 KO recipient; MKP5 to WT, MKP5 KO BM cells transferred to B6.SJL recipient) were stained with antibodies against CD45.1, CD45.2, CD11b, or TCRβ. The expression of CD45.1 and CD45.2 on CD11b$^+$ or TCRβ^+ cells was analyzed to determine the efficiency of bone marrow reconstitution. *B* and *C*, insulin tolerance test (*B*) and glucose tolerance test (*C*) were performed 12 to 15 weeks after reconstitution. Data are presented as mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

mice lacking MKP5 in their hematopoietic compartments developed insulin resistance (Fig. 5*B*), indicating that the insulin resistance in MKP5 KO mice was mainly mediated by their hematopoietic cells. Glucose tolerance tests showed that MKP5 KO mice reconstituted with WT mice bone marrow had little effect on glucose responsiveness (Fig. 5*C*). In contrast, reconstitution of WT mice with MKP5 KO bone marrow cells led to the development of glucose intolerance (Fig. 5*C*). Together, these results demonstrate that the development of insulin resistance and glucose intolerance in MKP5 KO mice is primarily caused by a defect in their hematopoietic cells.

MKP5 Deficiency Resulted in Increased Adipose Tissue Macrophage M1 Polarization—The interplay between macrophages and adipocytes in WAT is critical for inflammation and insulin resistance (21, 25–27). In line with increased macrophage infiltration in visceral WAT from MKP5 KO mice (Fig. 2*E*), we found significantly higher percentage of CD11b$^+$F4/80$^+$ cells in SVCs from KO visceral WAT than those from WT WAT (Fig. 6*A*), whereas macrophage infiltration in subcutaneous WAT was minimal in both WT and KO mice (data not shown). Moreover, higher percentage of F4/80$^+$CD11c$^+$ macrophages, the inflammatory macrophages in WAT (28), were identified in SVCs from KO than those from WT mice (Fig. 6*B*). The phenotypes of ATMs determine their function in insulin sensitivity. Classically activated or M1 ATMs promote insulin resistance, whereas alternatively activated or M2 macrophages are protective against the development of insulin resistance (26, 28). We thus studied the phenotypes of WT and KO ATMs. We found that MKP5 KO ATMs expressed significantly higher levels of M1 genes, including *IL-6*, *Nos2*, and *Tnfα* and significantly lower levels of M2 markers such as Mgl1, Mgl2, and *Mrc* than WT ATMs (Fig. 6, *C* and *D*). Collectively, these results demonstrated that the deficiency of MKP5 results in increased MCP-1 expression and increased infiltration of macrophages with M1 phenotype into the WAT.

Next, we generated WT and MKP5 KO BMDMs to further study the regulation of macrophage activation by MKP5 (Fig. 7*A*). Upon M1 polarization, as shown in Fig. 7*B*, MKP5 KO macrophages produced significantly higher amounts of IL-6, TNFα, and MCP-1 than WT cells. We also detected significantly increased TNFα production by MKP5 KO macrophages than WT cells in response to FFA stimulation (Fig. 7*C*).

We next co-cultured WT or MKP5 KO BMDMs with differentiated WT adipocytes to examine inflammatory cytokine gene expression and found significantly increased mRNA expression of IL-6 and MCP-1 in adipocyte-KO macrophage co-culture compared with adipocyte-WT macrophage co-culture (Fig. 7*D*). Supernatant from adipocyte-KO macrophage co-culture contained significantly higher amount of IL-6 than that from adipocyte-WT macrophage co-culture (Fig. 7*E*).

Discussion

Metabolic and immune systems are highly integrated and the proper function of one influences the other (4). Inflammation caused by activation of inflammatory signaling is causally associated with metabolic disorders, including obesity, insulin resistance, and type 2 diabetes (10, 11). Adipose tissue hosts the interaction of adipocytes with immune cells, critically modulating metabolic homeostasis (4–6). It is believed that obesity triggers macrophage infiltration into WAT to alter adipose tissue function, leading to systemic insulin resistance (21, 25, 28). Our study on MKP5 demonstrates that the deletion of MKP5 resulted in progressive development of metabolic disorders, including systemic insulin resistance, glucose intolerance, and obesity, associated with greatly increased infiltration of macrophages with M1 phenotype (Fig. 8). We further found that the hematopoietic compartment of MKP5-deficient mice were responsible for the development of their metabolic disorders. MKP5 appears to be required for the animals to deal with the condition where positive energy balance occurs. The expression of MKP5 is increased in WAT from aged mice (Fig. 1*A*), suggesting the requirement of this protein for maintaining metabolic homeostasis during aging-associated WAT expansion.

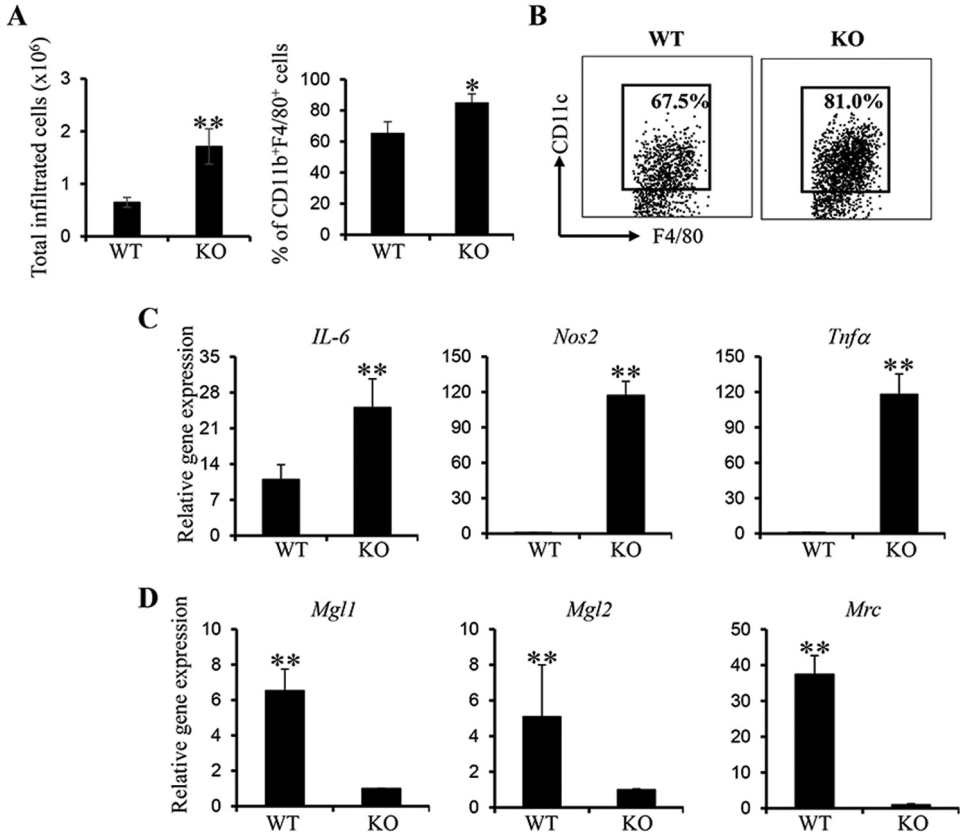

FIGURE 6. **Increased macrophage M1 activation in MKP5 KO adipose tissue.** Stromal vascular cells (SVCs) from 5-month-old male WT and MKP5 KO vWAT were stained with anti-CD45.2, anti-CD11b, and anti-F4/80 antibodies and were analyzed by FACS. Total infiltrated cells, percentage of $CD11b^+F4/80^+$ macrophages (*A*), and CD11c expression on the surface of $CD11b^+F4/80^+$ macrophages (*B*) were determined. The expressions of M1 genes (*C*) including *IL-6*, *Nos2*, and *Tnfa*, and M2 genes (*D*) including Mgl1, Mgl2, and *Mrc* in macrophages isolated from SVCs were determined by qPCR. The data shown are representative of three independent experiments with similar results and are presented as mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

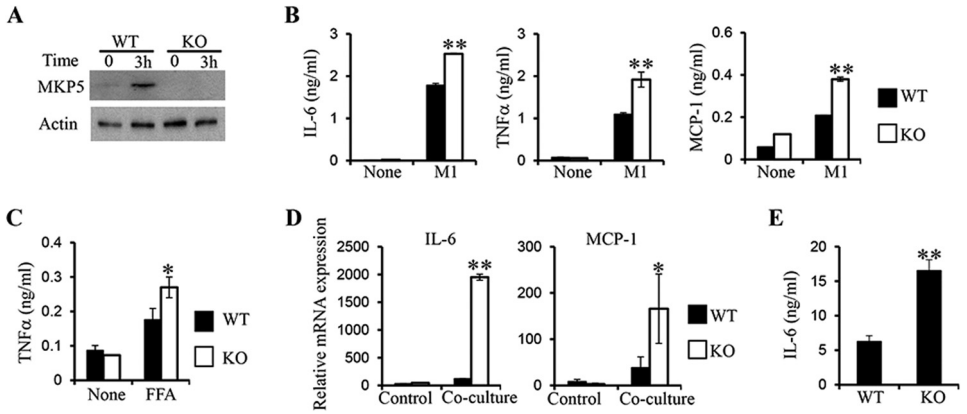

FIGURE 7. **MKP5-deficient macrophages are proinflammatory.** *A*, WT and KO BMDMs were stimulated with M1 condition to analyze MKP5 protein expression by Western blot. *B*, WT and KO BMDMs were stimulated with M1 condition. IL-6, TNFα, and MCP-1 concentrations in culture supernatants were determined by ELISA. *C*, WT and KO BMDMs were stimulated with or without 750 μM palmitate overnight. TNFα concentration in culture supernatants were determined by ELISA. *D*, WT and KO BMDMs were cultured alone or co-cultured with differentiated WT adipocytes derived from vWAT for 6 h. Cells were harvested for cDNA. IL-6 and MCP-1 expression was determined by qPCR. *E*, WT and MKP5 KO BMDMs were co-cultured with WT adipocytes overnight. IL-6 concentrations in supernatants were determined by ELISA. The data shown are representative of three independent experiments with similar results and are presented as mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

The development of visceral obesity in MKP5 KO mice is associated with systemic insulin resistance, glucose intolerance, and hyperinsulinemia (Figs. 1, 2*A*, and 3, *A* and *B*). It is known that adipose tissue from different anatomic sites has markedly different effects on metabolic outcomes, and visceral WAT in both humans and rodents is more closely correlated with obesity associated pathology than overall adiposity (29, 30). Importantly, visceral WAT in rodents appears to play a major role in modulating insulin action and glucose intolerance. Removal of visceral fat from rats dramatically improved peripheral and hepatic insulin action without causing detectable changes in body weight and composition (30, 31), indicating factors that are selectively expressed in visceral WAT are important in the pathogenesis of insulin resistance and

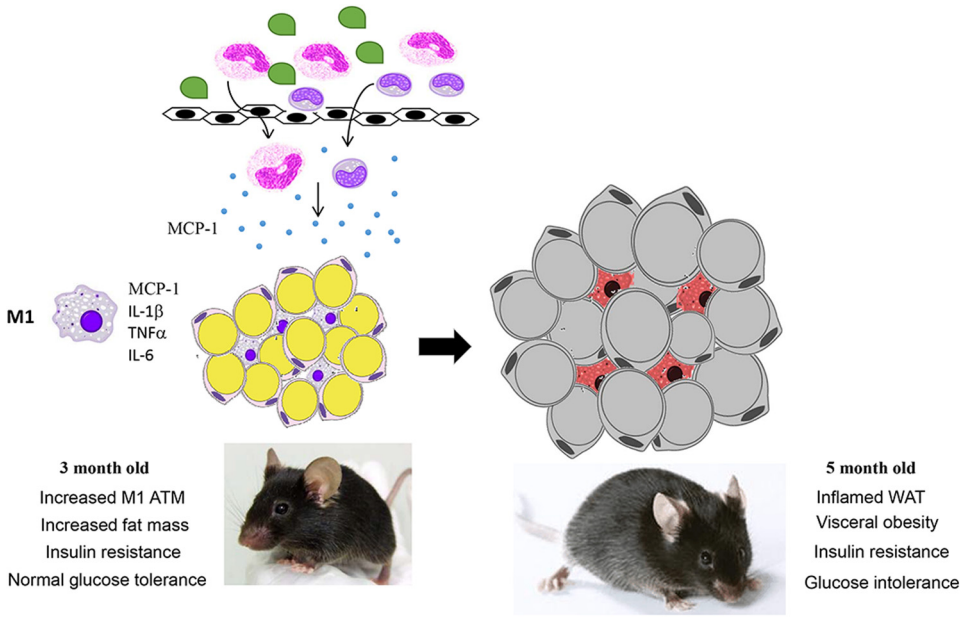

FIGURE 8. **Development of obesity and obesity-associated metabolic disorders in MKP5-deficient mice.** Increased MCP-1 expression in WAT in MKP5 KO mice results in the recruitment of monocytes from circulation into WAT and differentiate into M1 macrophages, which interfere with adipose tissue function, leading to the development of obesity-associated metabolic disorders in MKP5 KO mice.

the cross-talk between fat depots and distant sites. Therefore, it is not surprising that the metabolic disorders observed in MKP5 KO mice are mainly caused by the dysfunction of MKP5-deficient visceral WAT, and MKP5 is an important factor mediating the cross-talk between visceral WAT and distant sites.

Obesity is not necessary to cause insulin resistance. It has been shown that the deficiency of GPR105, a G protein-coupled receptor, protected mice from the development of insulin resistance and glucose intolerance associated with reduced inflammation without affecting the development of obesity in response to high fat diet (32). Improved insulin sensitivity was observed in T-bet KO mice despite their development of visceral obesity (33). Treatment of mice with omega-3 fatty acid, an anti-inflammatory agent, resulted in reduced inflammatory macrophage infiltration in WAT and improved insulin signaling in high fat diet-induced obesity (34). It is recognized that ATMs are the major contributor to inflammation and insulin resistance (7). The recruitment of macrophages into WAT in obesity and the phenotypic switch of ATMs from the alternative (M2) activation to the proinflammatory (M1) activation have been shown to be necessary for the development of insulin resistance and the related metabolic disorders (21, 28). Understanding the mechanisms that regulate both the recruitment and the proinflammatory activation of ATMs will be beneficial for the development of therapeutic methods for those metabolic disorders. Recently, Han *et al.* (35) found that macrophage-specific deletion of JNK resulted in reduced WAT macrophage infiltration and impaired macrophage M1 activation, which protected the mice from the development of high fat diet-induced insulin resistance, supporting the critical role of WAT macrophages in metabolic homeostasis in obesity. Our data clearly demonstrate that MKP5 regulates WAT macrophage infiltration associated with its regulation on MCP-1 and ATM M1 activation. It appears that macrophage infiltration into WAT precedes the development of obesity in MKP5 KO mice (Fig. 2*E*). Therefore, our study revealed that MKP5 is an essential control measure for WAT macrophage infiltration and M1 activation, playing an important role in WAT homeostasis.

Previously, we have demonstrated that MKP5 is a JNK phosphatase (16). JNK MAPK pathway has been shown to have critical roles in inflammation, obesity, and insulin resistance (9, 36). Surprisingly, we found that the activation p38 MAPK, but not JNK or ERK (Fig. 4*C*), was enhanced in the KO WAT, indicating that MKP5 regulating MAPK activation in a tissue-specific manner, which could be explained by the compensation of other MKP members in that tissue or by the presence of regulatory mechanisms such as scaffold proteins. p38 MAPK is an important regulator of inflammatory responses (37). The activation of p38 was enhanced in insulin-resistant tissues from type 2 diabetic patients (38). Recently, p38 was found to play an important role in the induction of hepatic insulin resistance due to mitochondrial dysfunction and mice deficient in p38δ were protected against high fat diet-induced insulin resistance and glucose intolerance (39, 40). It is possible that enhanced p38 activation in MKP5 KO WAT contributes to the development of insulin resistance. The physiological activators of p38 in the WAT from MKP5 KO mice are to be determined.

In conclusion, our study demonstrated that MKP5 plays an important role in the prevention of adipose tissue inflammation and is required for appropriate insulin responsiveness in obesity and thus prevents the development of insulin resistance and metabolic disorders. Our study has further supported a role of inflammation in type 2 diabetes and sheds new light on the pathogenesis of this widespread disease.

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