Supplementary Information

MATERIALS AND METHODS

Cell culture and adipocyte differentiation assay

3T3-L1 cells were kindly provided by professor Jae-woo Kim (Yonsei University). 3T3-L1 cells were maintained and differentiated as previously described (1). Briefly, 3T3-L1 cells were grown to post-confluence with DMEM containing 10% bovine serum. On day 0 post-confluence, 3T3-L1 cells were incubated with DMEM containing 10% fetal bovine serum (FBS), insulin (1μg/ml), isobutylmethylxanthine (520 μM), and dexamethasone (1 μM). After 2 days, media was replaced with DMEM containing 10% FBS and insulin (1 μg/ml). On day 4, media was replaced with DMEM, supplemented with only 10% FBS.

Cell viability assay

3T3-L1 cells were plated in 12-well plates and incubated until confluence. Then, 3T3-L1 cells were treated with kahweol for 48 h. Cell viability was measured using Ez-Cytox (Daeil Lab), according to the manufacturer's protocol (2, 3). This experiment was performed in triplicate.

Oil Red O staining

Differentiated 3T3-L1 cells were incubated with 10% formalin for 10 min and washed with distilled water. Then, cells were stained with Oil Red O (ORO) in 60% isopropanol. Stained ORO was eluted with 100% isopropanol and measured at OD_{500}.

Western blot analysis

Cell lysate extractions were prepared with RIPA buffer (1% Triton X-100; 1% sodium deoxycholate; 0.1% sodium dodecyl sulfate; 150 mM NaCl; 50 mM Tris-HCl, pH 7.5; and 2 mM EDTA, pH 8.0) as previously described (1). Primary antibodies (C/EBPα, PPARγ, FABP4, FASN, p-AMPK, AMPK, p-

AKT, AKT, p-JAK2 and JAK2 from Santa Cruz Biotechnology; p-ACC, ACC, p-ERK1/2 and ERK1/2 from Cell Signaling) were used for detection using a FUSION SOLO S (Vilber) detector according to the manufacturer's directions (4-6). The normalization control was anti-β-actin (Santa Cruz Biotechnology).

RNA isolation and real-time PCR analysis

RNA was prepared using the RNA-lysis reagent (5 PRIME) as previously described (7). cDNA (1 μg) was synthesized from RNA using qPCR RT master mix (TOYOBO). The following primers were used: PPARγ: F_5′-agggcgatcttgacaggaaa-3′ and R_5′-cgaaactggcacccttgaaa-3′; C/EBPα: F_5′-gacatcagcgcctacatcga-3′ and R_5′-tcggctgtgctggaagag-3′; FABP4: F_5′-catcagcgtaaatggggatt-3′ and R_5′-tcgactttccatcccacttc-3′; FASN: F_5′-tgggttctagccagcagagt-3′ and R_5′-accaccagagaccgttatgc-3′; β-actin: F_5′-ggctgtattcccctccatcg-3′ and R_5′-ccagttggtaacaatgccatgt-3′. Real-time PCR was performed using SYBR Green Master Mix (TOYOBO) with an instrument from Applied Biosystems (8-10).

Transfection of small interfering RNA

3T3-L1 cells were plated in a 6-well plate and incubated for 24 h. 3T3-L1 cells were transfected with mouse AMPKa1 siRNA at 50 nM (GenePharma) using the Lipofectamine RNAiMAX (Invitrogen), according to the manufacturer's protocol (11, 12). After 24 h, media was replaced with DMEM containing 10% bovine serum. On the next day, differentiation of 3T3-L1 cells was induced in DMEM containing 10% FBS, insulin (1 μg/ml), isobutylmethylxanthine (520 μM), and dexamethasone (1 μM).

Glucose uptake assay

3T3-L1 cells were plated in a 12-well plate and incubated for 4 day. 3T3-L1 cells were incubated with DMEM containing insulin, isobutylmethylxanthine and dexamethasone. After 2 days, media were replaced with DMEM containing kahweol or metformin. After 48 h, glucose uptake was quantitated

using a glucose assay kit (Eton Bioscience), according to the manufacturer's protocol. This experiment was performed in triplicate.

Glucose tolerance test (GTT)

For glucose tolerance tests, wild-type male C57BL/6 mice were fasted overnight. After 15 h, fasting blood glucose levels were measured using a glucometer. Then, glucose solution (1 g/kg) was administered by intraperitoneal injection. Blood glucose levels were measured at 15, 30, 60, 90, and 120 minutes. Animal study was approved by the Yonsei University Health System Institutional Animal Care and Use Committee.

Statistical analysis

We employed unpaired *t*-tests to analyze comparisons between two groups. Statistical analysis was performed using Prism 5. P values < 0.05 were considered to be significant.

Reference

1. Baek, J. H., Kim, S. J., Kang, H. G. et al (2015) Galectin-3 activates PPARgamma and supports white adipose tissue formation and high-fat diet-induced obesity. Endocrinology 156, 147-156.
2. Choi, S. W., Song, J. K., Yim, Y. S., Yun, H. G. and Chun, K. H. (2015) Glucose deprivation triggers protein kinase C-dependent beta-catenin proteasomal degradation. J Biol Chem 290, 9863-9873.
3. Lee, H. W., Jang, K. S., Choi, H. J., Jo, A., Cheong, J. H. and Chun, K. H. (2014) Celastrol inhibits gastric cancer growth by induction of apoptosis and autophagy. BMB Rep 47, 697-702.
4. Kim, S. J., Lee, H. W., Gu Kang, H. et al (2014) Ablation of galectin-3 induces p27(KIP1)-dependent premature senescence without oncogenic stress. Cell Death Differ 21, 1769-1779.
5. Kim, S. J., Choi, I. J., Cheong, T. C. et al (2010) Galectin-3 increases gastric cancer cell motility by up-regulating fascin-1 expression. Gastroenterology 138, 1035-1045.
6. Kim, S. J., Lee, H. W., Baek, J. H. et al (2016) Activation of nuclear PTEN by inhibition of Notch signaling induces G2/M cell cycle arrest in gastric cancer. Oncogene 35, 251-260.
7. Ahn, Y. H., Yi, H., Shin, J. Y. et al (2012) STAT3 silencing enhances the efficacy of the HSV.tk

suicide gene in gastrointestinal cancer therapy. Clin Exp Metastasis 29, 359-369.

8. La, S. H., Kim, S. J., Kang, H. G., Lee, H. W. and Chun, K. H. (2016) Ablation of human telomerase reverse transcriptase (hTERT) induces cellular senescence in gastric cancer through a galectin-3 dependent mechanism. Oncotarget.
9. Cho, Y., Lee, H. W., Kang, H. G., Kim, H. Y., Kim, S. J. and Chun, K. H. (2015) Cleaved CD44 intracellular domain supports activation of stemness factors and promotes tumorigenesis of breast cancer. Oncotarget 6, 8709-8721.
10. Kim, S. J., Wang, Y. G., Lee, H. W. et al (2014) Up-regulation of neogenin-1 increases cell proliferation and motility in gastric cancer. Oncotarget 5, 3386-3398.
11. Wang, Y. G., Kim, S. J., Baek, J. H., Lee, H. W., Jeong, S. Y. and Chun, K. H. (2012) Galectin-3 increases the motility of mouse melanoma cells by regulating matrix metalloproteinase-1 expression. Exp Mol Med 44, 387-393.
12. Kim, S. J., Shin, J. Y., Lee, K. D. et al (2012) MicroRNA let-7a suppresses breast cancer cell migration and invasion through downregulation of C-C chemokine receptor type 7. Breast Cancer Res 14, R14.