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Osteopontin-induced brown adipogenesis from white preadipocytes through a PI3K-AKT dependent signaling

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ABSTRACT

Recent studies have shown that OPN (osteopontin) plays critical roles in cell survival, differentiation, bio-mineralization, cancer and cardiovascular remodeling. However, its roles in the differentiation of brown adipocytes and the underlying mechanisms remain unclear. Therefore, the aim of this study was to investigate the roles of OPN in the brown adipogenesis and the underlying mechanisms. It was shown that the OPN successfully induced the differentiation of 3T3-L1 white preadipocytes into the PRDM16^{+} (PRD1-BF1-RIZ1 homologous domain containing 16) and UCP-1^{+} (uncoupling protein-1) brown adipocytes in a concentration and time-dependent manner. Also, activation of PI3K (phosphatidylinositol 3-kinase)-AKT pathway was required for the OPN-induced brown adipogenesis. The findings suggest OPN plays an important role in promoting the differentiation of the brown adipocytes and might provide a potential novel therapeutic approach for the treatment of obesity and related disorders.

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1. Introduction

Adipose tissue plays an important role in metabolic homeostasis, and consequently, change in its functions may lead to metabolic disorders, most notably type 2 diabetes mellitus (T2D), atherosclerosis (AS), hypertension, and cancers [1–3]. White adipocyte (WA) (predominantly existing in white adipose tissue) and brown adipocyte (BA) (predominantly in brown adipose tissue) differ in many aspects such as origin, distribution, morphology and functions [4,5]. The WA stores triglyceride (TG) and contributes to obesity, while the BA 'burns' fat through fatty acid-β-oxidation and contributes to weight loss [6,7]. Therefore, investigating the mechanisms underlying BA differentiation and brown adipogenesis would provide a novel strategy or avenue for prevention and treatment of obesity and related disorders.

Although WA and BA originate from the same mysenchymal stem cells, they differ in morphology and molecular phenotype [8,9]. Morphologically, mature BA is a multilocular cell abundant in lipid and mitochondrium, while mature WA is a unilocular cell deficient in the mitochondrium [10]. PRDM16 (PRD1-BF1-RIZ1 homologous domain containing 16) is recently identified as an early determinator of the brown adipogenesis, and UCP-1 (uncoupling protein-1) is generally considered as a BA specific protein marker [11,12]. Consequently, the BA is characterized as a PRDM16^{+}/UCP-1^{+} adipocyte, and the WA as an angiotensin^{+}/resistin^{+} cell. Therefore, these markers are commonly used to identify BA or WA [13,14]. Numerous studies have demonstrated that both cold exposure and lasting excitation of sympathetic nerves robustly promote brown adipose conversion from white preadipocytes, and this adaptive brown adipogenesis is beneficial to defend colds and bad states [15–17]. Given above, promotion of BA differentiation can be possibly used to fight against obesity. Unfortunately, transcriptional regulation mechanisms underlying the BA differentiation remain largely unclear.

Abbreviations: AS, atherosclerosis; BA, brown adipocyte; iOPN, intracellular OPN; OPN, osteopontin; PI3K, phosphatidylinositol 3-kinase; PRDM16, PRD1-BF1-RIZ1 homologous domain containing 16; UCP-1^{+}, uncoupling protein-1; SPP-1, secreted phosphoprotein-1; sOPN, secreted OPN; T2D, type 2 diabetes mellitus; TG, triglyceride; WA, white adipocyte.

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OPN (Osteopontin), known as SPP-1 (secreted phosphoprotein-1), are extensively expressed in diverse cells such as osteoblasts, endothelial cells, macrophages, T cells, hepatic stellate cells, and smooth muscle cells [18]. Recent studies have shown that OPN is implicated in many critical pathophysiological processes including bio-mineralization, differentiation, carcinogenesis and cardiovascular remodeling, etc [19,20]. However, its roles and the mechanisms underlying the differentiation of BA remain elusive. Therefore, the aim was to investigate the roles of OPN in brown adipogenesis and to explore the mechanisms underlying the differentiation.

2. Materials and methods

2.1. Materials

All chemicals were acquired commercially. High-glucose Dulbecco's modified Earle's medium (H-DMEM) was purchased from Gibco-BRL (NY, USA). Antibodies against OPN, PRDM16, UCP-1, AKT and β-actin (Santa Cruz Biotechnology, CA, USA), Phospho-AKT (serine473), and αvβ3 integrin (Cell Signaling Technology, MA, USA) were freshly prepared. OPN ELISA kit, KpnI and Xba were acquired from R&D Systems (MN, USA) and New England Biolabs (London, UK), respectively. Total RNA was isolated with QIAzol lysis reagent (Qiagen, Valencia, CA, USA). AKT inhibitor Ⅳ and LY294002 (Sigma—Aldrich, St. Louis, MO, USA) were dissolved in DMSO. All other chemicals were purchased from Sigma—Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.2. DNA constructs and cell transfection

Flag-tagged OPN was amplified by PCR using full length OPN as a template, and sub-cloned into the pCMV5 vector as our previously described [21]. The sequences used here are as follows: sense (5′-GGGGTACCTATGAGATTGGCAGTGAT-3′) and antisense (5′-GCTCTAGACGCCTCTTCTTTAGTTGAC-3′). In addition, cell transfection was conducted following the manufacturer's instructions (Invitrogen). Briefly, 3T3-L1 cells were plated in the 6-well plate at 2–8 × 10^5 cells/well and were allowed to grow to 95% confluence. The cells were washed twice with the serum-free DMEM and transfected with 2 μg, 4 μg, 6 μg pCMV5-flag-OPN vector, respectively, and 4 μg pCMV5-flag empty vector was used as a control by Lipofectamine 2000 (Invitrogen). The cells were cultured at 37 °C in the 95% O_2–5% CO_2 humidified atmosphere. Six hours after transfection, the serum-free DMEM was replaced by the complete DMEM containing 10% FBS. Forty-eight hours after transfection, the cells were harvested and detected by Western blots.

2.3. Cell culture and differentiation

The 3T3-L1 white preadipocytes were obtained from the American Type Culture Collection (ATCC, USA). The 3T3-L1 cells were cultured as our previously described [22]. The method used for *In vitro* differentiation was adopted from the report by Tseng et al., 2008 [23]. Briefly, the 60% confluent 3T3-L1 white preadipocytes were incubated in the serum-free DMEM/F12 medium

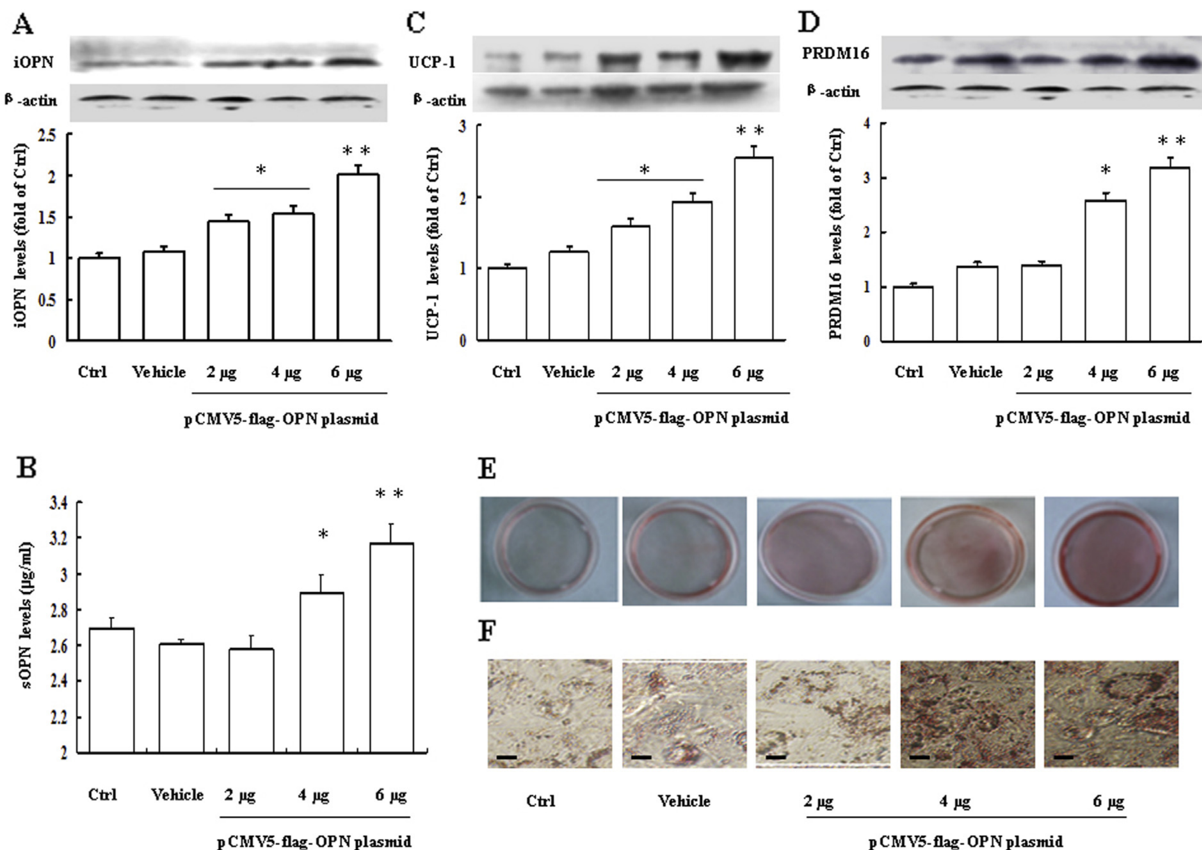

Fig. 1. Concentration-effect of OPN-induced brown adipogenesis. The expression of iOPN (A), UCP-1 (C), PRDM16 (D), and morphologic elements (oil-red O staining, E and F) of the transfected 3T3-L1 white preadipocytes with 2 μg, 4 μg, 6 μg pCMV5-flag-OPN vector, or 4 μg pCMV5-flag empty vector (Vehicle), and untransfected (Ctrl) 3T3-L1 white preadipocytes following differentiation for 6 days are shown. The sOPN (B) was measured from the supernatant following differentiation. Data are expressed as mean ± S.E.M. of 4 independent experiments. **P* < 0.05, ***P* < 0.01, vs. Ctrl or Vehicle. Scale bars, 200 μm iOPN, intracellular OPN; sOPN, secreted OPN.

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