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Research Article

Apolipoprotein E promotes lipid accumulation and differentiation in human adipocytes

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ABSTRACT

Several studies in mice indicate a role for apolipoprotein E (APOE) in lipid accumulation and adipogenic differentiation in adipose tissue. However, little is yet known if APOE functions in a similar manner in human adipocytes. This prompted us to compare lipid loading and expression of adipocyte differentiation markers in APOE-deficient and control adipocytes using the differentiated human mesenchymal stem cell line hMSC-Tert as well as primary human and mouse adipocytes as model systems. Differentiated hMSC-Tert were stably transduced with or without siRNA targeting APOE while murine adipocytes were isolated from wild type and Apoe knockout mice. Human APOE knockdown hMSC-Tert adipocytes accumulated markedly less triglycerides compared to control cells. This correlated with strongly decreased gene expression levels of adipocyte markers such as adiponectin (*ADIPOQ*) and fatty acid binding protein 4 (*FABP4*) as well as the key transcription factor driving adipocyte differentiation, peroxisome proliferator activator receptor gamma (*PPARG*), in particular the PPARG2 isoform. Similarly, differentiation of murine Apoe-deficient adipocytes was characterized by reduced gene expression of *Adipoq*, *Fabp4* and *Pparg*. Interestingly, incubation of APOE-deficient hMSC-Tert adipocytes with conditioned media from APOE3-overexpressing adipocytes or APOE-containing Very Low Density Lipoprotein (VLDL) partially restored triglyceride accumulation, but were unable to induce adipocyte differentiation, as judged by expression of adipocyte markers. Taken together, depletion of endogenous APOE in human adipocytes severely impairs lipid accumulation, which is associated with an inability to initiate differentiation.

1. Introduction

The development of metabolic disorders such as insulin resistance and type 2 diabetes is associated with an increase of adipose tissue mass, either due to hypertrophy of existing adipocytes or alternatively, increased adipogenic differentiation of precursor cells [1–6]. In adipocytes, besides regulating the expression of adipocyte-specific genes such as adiponectin (*ADIPOQ*) and fatty acid binding protein 4 (*FABP4*), peroxisome proliferator activated receptor γ (*PPARG*) is involved in the transcriptional up-regulation of apolipoprotein E (*APOE*) [7]. APOE has been linked to various cellular processes and most studies focused on the role of APOE as a structural component of lipoproteins. In this context, APOE regulates intracellular and intravascular lipoprotein metabolism by virtue of its binding capacity to several cell surface lipoprotein receptors [8,9]. For instance, APOE mediates the internalization of postprandial chylomicron remnants (CR) into hepatocytes via low density lipoprotein receptor (LDLR)- and LDLR-related protein 1 (LRP1)-mediated endocytosis [10]. In addition, APOE has recently been linked to energy homeostasis [11] and accumulation of triglycerides [12] in mouse adipocytes. Along these lines, Apoe-deficient mice ($Apoe^{-/-}$) are leaner [13–15] and exhibit better glucose tolerance and insulin sensitivity as their wild type littermates on both normal or high-fat diet [16,17]. In addition, genetically obese mice on an Apoe-deficient background are protected from obesity and insulin resistance [16]. Given the key role of APOE in lipoprotein internalization, the suppressed body weight gain and fat accumulation in Apoe-deficient mice is most likely explained by impaired delivery of liver-derived VLDL into adipose tissue. In

Abbreviations: ADIPOQ, adiponectin; APOE, apolipoprotein E; CR, chylomicron remnant; FABP4, fatty acid binding protein 4; FCS, fetal calf serum; FFA, free fatty acids; LDLR, low density lipoprotein receptor; LRP1, LDLR-related protein 1; MSC, mesenchymal stem cell; PPARG, peroxisome proliferator activated receptor gamma; TG, triglyceride; VLDL, very low density lipoprotein

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fact, APOE interacts with the VLDL receptor (VLDLR) on the cell surface of adipocytes to facilitate hydrolysis of triglycerides (TG) by lipoprotein lipase (LPL) [18]. In line with these findings, adipocyte-derived APOE promotes TG uptake *in vitro* and lack of endogenous APOE led to a marked defect in TG uptake from exogenous VLDL in mouse model systems [19].

Interestingly, VLDL from wild type, but not from Apoe-knockout mice can induce adipogenesis in murine 3T3-L1 and bone marrow stromal cells [20], indicating that lipid accumulation and differentiation are closely linked in mouse adipocytes. Furthermore, accumulating evidence suggests that-as Apoe modulates glucose tolerance and insulin sensitivity in mouse adipocytes–*Apoe* expression might impact on adipocyte differentiation. Importantly, all of the data on Apoe described above is predominantly derived from mouse adipocytes, while no comparable data is yet available that would validate these findings in human adipocytes. As a first step to overcome this difficulty, we lately established the human mesenchymal stem cell line hMSC-Tert as a new model for human adipocytes [21]. This cell model resembles features close to terminally differentiated human adipocytes with regard to morphology, marker expression and metabolic function. To detect if there was an APOE-dependent association of lipid uptake and differentiation in human adipocytes, we analyzed lipid accumulation and gene expression of adipocyte markers in *APOE*-deficient adipocytes derived from a differentiated human hMSC-Tert cell line [22].

Knockdown of endogenous APOE lead to drastically impaired lipid accumulation and adipogenesis. Interestingly, supplementation with exogenous APOE only partially restored lipid accumulation, without significantly enhancing adipocyte differentiation marker expression in APOE-deficient hMSC-Tert adipocytes. These findings suggest dual and possibly associated functions of endogenous APOE for lipid accumulation and differentiation of human adipocytes.

2. Materials and methods

2.1. Cell culture and differentiation

hMSC-Tert cells were cultured in DMEM high glucose (with GlutaMAX I, 4500 mg/L D-glucose, sodium pyruvate), 100 U/mL penicillin/streptomycin and 10% fetal calf serum (FCS, Biowithaker) at 37 °C, 5% CO_2 and 95% humidity. Adipogenic differentiation was induced by culturing cells in differentiation medium for 14 days as described [21].

Primary cultures of human preadipocytes from visceral adipose tissues were obtained from PromoCell. Mouse preadipocytes were isolated from epididymal adipose tissues obtained from wild type C57BL/6J (WT) and *Apoe*-deficient mice. In brief, after removal of blood vessels and fibrous material, tissue samples were minced and digested in PBS containing 2 mg/mL collagenase type I (Worthington Biochemical Corp.) for 60 min at 37 °C. The suspension was then filtered through a 70 μm nylon mesh (BD Falcon), and cells were centrifuged for 10 min at 1000 rpm. The cell pellet was washed with DMEM/F-12 containing 20% FCS, passed through a 40 μm nylon mesh (BD Falcon) and centrifuged for 5 min at 1500 rpm. The sedimented cell fraction was incubated with erythrocyte lysis buffer containing 154 mmol/L NH4Cl, 10 mmol/L KHCO3, and 0.1 mmol/L EDTA (Merck) for 10 min at room temperature (RT). After centrifugation for 5 min at 1500 rpm, the cells were resuspended in DMEM/F-12 containing 20% FCS and seeded into 24-well plates. Adipose differentiation of human and mouse primary preadipocytes was induced with serum-free DMEM/F-12 containing 33 μmol/L biotin, 17 μmol/L pantothenate, 1 nmol/L triiodothyronine, 100 nmol/L dexamethasone, 500 nmol/L insulin, 1 μmol/L rosiglitazone (Cayman), and 0.25 μmol/L isobutyl-methylxanthine (day 1–3). Differentiation medium was replaced three times a week over a period of 10 days. Animal care and experimental procedures were performed with approval from the animal care committees of the University Medical Center Hamburg-Eppendorf.

2.2. Supplementation of adipogenic differentiation with APOE3-conditioned media

Conditioned media enriched with APOE was obtained from a stable human APOE3-overexpressing hMSC-Tert cell line, which secreted highly elevated levels of APOE (hMSC-Tert-h-APOE3-Lenti; see Supplemental Fig. 1). For supplementation experiments, equal volumes of APOE3-conditioned and fresh differentiation media were used for differentiation as described above. hMSC-Tert-shControl and hMSC-Tert-shAPOE cells were then differentiated in the presence or absence of APOE3-conditioned media for the duration of 14 days.

2.3. Oil Red O staining

Cells were fixed in 4% paraformaldehyde for 30 min at RT, washed with PBS and 60% isopropanol. Neutral lipids were stained with 0.3% Oil Red O in 60% isopropanol for 10 min followed by washes with water. Phase contrast was analyzed by conventional microscopy. For quantification Oil Red O was washed out with 100% isopropanol and collected. OD540 of the lipid dye was measured using Biotrak II Plate Reader (Amersham Biosciences).

2.4. RNA isolation, cDNA synthesis and quantitative real-time PCR

Total RNA was extracted using the TRIzol Reagent (Invitrogen) and purified with the NucleoSpin RNAII (Macherey-Nagel). The RNA purity and concentration was determined using the NanoDrop ND-1000 (PeqLab). Subsequent cDNA synthesis was performed with 500 ng of RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following the manufacturer's instructions. Relative mRNA levels were determined by quantitative RT-PCR (qRT-PCR) using the TaqMan Gene Expression Assay Technique and the 7900HT Fast Real-Time PCR System (Applied Biosystems). The following TaqManAssay-on-Demand primer sets were purchased from Invitrogen: *mAdipoq* (Mm00456425_m1); *mApoe* (Mm01307193_g1); *mFabp4* (Mm00445880_m1); *mPparg* (Mm00440945_m1); *mSlc2a4* (Glut4, Mm01245502_m1); *mTbp* (Mm00446973_m1); hADIPOQ (Hs00605917_m1); hAPOE (Hs00171168_m1); hFABP4 (Hs00609791_m1); hPPARG (Hs00234592_m1); hSLC2A4 (Hs00168966_m1); hTAF1 (Hs00270322_m1). Gene of interest cycle thresholds (Cts) were normalized to TATA-box binding protein (*Tbp*) house keeper levels by the ΔΔCt method and displayed as relative copies per *Tbp* or relative expression normalized to experimental control groups.

2.5. Protein isolation, western blotting and ELISA

Total cell protein from hMSC-Tert cells was prepared in 50 mM Tris pH 8.0 with 80 mM NaCl, 2 mM $CaCl_2$, 1% Triton X-100, 1 mM Na_3VO_4, protease inhibitors (Complete Mini, Roche). Protein concentration was measured using the Lowry Protein Assay. Proteins were separated by SDS-PAGE in 4–12% gradient gels (Invitrogen), transferred to nitrocellulose (Schleicher&Schuell) and probed with specific antibodies against PPARG (81B8, Cell Signaling Technologies), APOE (Dako) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Acris). The appropriate secondary horseradish peroxidase (HRP)-labeled antibodies were purchased from Jackson