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Role of leukotriene B_4 receptor signaling in human preadipocyte differentiation

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ABSTRACT

We investigated the role of leukotriene B_4 (LTB_4)–leukotriene receptor (BLT) signaling in preadipocyte differentiation into mature adipocytes. Blockade of BLT signaling by treatment with lipoxygenase inhibitors, a BLT antagonist, and small interfering RNAs for BLTs in human and mouse preadipocytes isolated from adipose tissues showed acceleration of differentiation into mature adipocytes. DNA microarray analysis revealed regulation of transforming growth factor, beta-induced 68 kDa (TGFBI) expression through the BLT signaling pathway during adipocyte differentiation. Knockdown of TGFBI also showed acceleration of preadipocyte differentiation. The LTB_4–BLT signaling pathway may negatively regulate preadipocyte differentiation via induction of TGFBI expression as a rate-limiting system to control adipocyte differentiation.

1. Introduction

The involvement of various inflammatory mediators such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6) in obesity that is closely related to insulin resistance has been reported [1–3]. One of the most important organs on obesity and insulin resistance is thought to be adipose tissue, because adipocytes in adipose tissue generate various adipocytokines that play important roles in the onset of metabolic syndrome [1–3].

Leukotrienes (LTs) such as leukotriene B_4, C_4, and D_4 (LTB_4, LTC_4, and LTD_4, respectively) that are generated through lipoxygenase (LOX) pathways are well-known to induce various inflammatory and allergic reactions such as leukocyte activation, capillary permeability, and bronchial contraction [4,5]. LTB_4 binds to specific receptors, BLT1 and BLT2, to activate the signaling pathways [6,7]. LTs have been reported to be involved in the proliferation of various cell types such as epithelial, endothelial and mesangial cells [8,9]. In addition, we have reported that LTB_4 controls immature neural stem cell proliferation and differentiation via the BLT signaling pathway [10]. Therefore, LTB_4 and its signaling pathway could be involved in various cell proliferations and differentiations. However, there are few reports about the role of LTs in adipocyte differentiation. Furthermore, most of reported papers about the adipocyte differentiation have used mouse fibroblastic 3T3-L1 cells, but not human preadipocytes. Therefore, the exact role of the LTB_4 signaling pathway in adipocyte differentiation of human preadipocytes is still unclear.

In this study, we investigated the role of LTB_4 and its receptor signaling pathway in human adipocyte differentiation and the potential mechanisms.

2. Material and methods

2.1. Reagents and antibodies

Insulin (INS), dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX) and rosiglitazone (ROSI) were purchased from Sigma Japan (Tokyo, Japan). LT synthetase, lipoxygenase (LOX) inhibitor, nordehydroguaiaretic acid (NDGA), and 5-LOX specific inhibitor, AA-861, were also purchased from Sigma Japan (Tokyo, Japan). ONO-4057, a specific BLT antagonist, was a kind gift from ONO Pharmaceutical Co. Ltd. (Osaka, Japan). Anti-BLT1 and -BLT2 polyclonal antibodies were purchased from Cayman Chemicals (Ann Arbor, MI, USA). An anti-TGF beta induced, 68 kDa (TGFBI,

Abbreviations: LT, leukotriene; LOX, lipoxygenase; TGFBI, transforming growth factor beta-induced 68 kDa; INS, insulin; DEX, dexamethasone; IBMX, 3-isobutyl-methylxanthine; ROSI, rosiglitazone; NDGA, nordehydroguaiaretic acid; TNFα, tumor necrosis factor alpha; IL-6, interleukin 6.

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synonym; Beta ig-h3) polyclonal antibody was purchased from Proteintech (Proteintech Group, Inc., Chicago, IL).

2.2. Cell culture and induction of adipocyte differentiation

Two types of human preadipocytes isolated from subcutaneous adipose tissue or visceral adipose tissue (Poietics Human Preadipocytes) were purchased from LONZA (LONZA Walkersville, Inc., Walkersville, MD). Study protocols for using human cells were approved by the Ethics Committee of Yokohama City University School of Medicine and Osaka University Graduate School of Dentistry.

Mouse preadipocytes isolated from epididymal adipose tissue were purchased from Primary Cell Ltd. (Sapporo, Japan) [11,12].

Cell culture and induction of differentiation of preadipocytes were performed essentially according to the method described previously (Fig. 1A) [11,12]. Briefly, two human preadipocytes isolated from subcutaneous or visceral adipose tissue and mouse preadipocytes were cultured in preadipocyte basal medium-2 (PBM-2, LONZA Walkersville) supplemented with 10% fetal bovine serum,

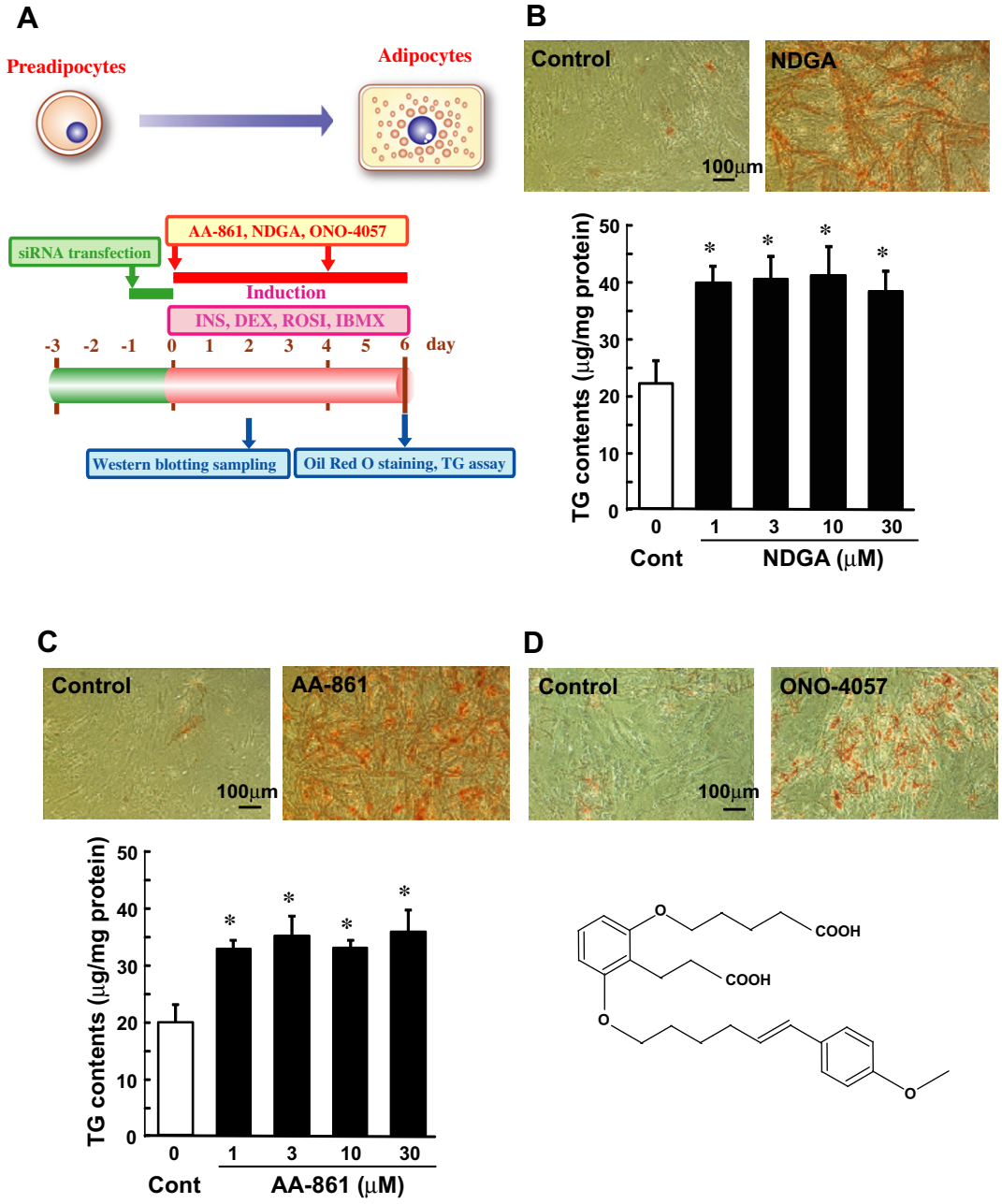

Fig. 1. Effects of LOX inhibitors and the BLT antagonist on human preadipocyte differentiation. (A) Schematic protocol of the preadipocyte differentiation. (B and C) Effects of NDGA (LOX inhibitor, B), or AA-861 (5-LOX inhibitor, C), on lipid accumulation in human preadipocytes isolated from subcutaneous adipose tissues. Human preadipocytes were treated with NDGA or AA-861 for 6 days. Then, accumulation of triacylglycerol (TG), a marker of lipid accumulation (right panel), in matured adipocytes was measured and expressed as TG contents (μg/mg protein). Each column represents the mean ± SEM from 3–5 independent experiments. $^{*}P < 0.05$ vs. vehicle control. Left panel represents typical photographs of differentiated adipocytes derived from human preadipocytes treated with NDGA, AA-861 or vehicle for 6 days. Cells were stained with Oil Red O method to visualize lipid accumulation. (D) Effect of ONO-4057, a specific BLT antagonist, on lipid accumulation in human preadipocytes. Left panel represents structure of ONO-4057, and right panel represents typical photographs of differentiated adipocytes from human preadipocytes treated with ONO-4057 (30 μM) or vehicle-only.