Peroxisome Proliferator–Activated Receptor-γ–Mediated Signaling Regulates Mitochondrial Energy Metabolism in Human Hair Follicle Epithelium


TO THE EDITOR

Peroxisome proliferator–activated receptors (PPARs) are ligand-activated transcription factors belonging to the family of nuclear hormone receptors. They include three different isoforms: namely PPAR-α, PPAR-β/δ, and PPAR-γ, and have many important roles in the regulation of a large number of physiological processes, including cell proliferation, differentiation, and inflammatory responses (Sertznig et al., 2008). Due to their prominent expression in human skin and its appendages, there is a growing interest in PPARs in human skin biology and pathology (Dozsa et al., 2016; Ramot et al., 2015; Ruzehaji et al., 2016; Wallmeyer et al., 2015; Yin and Smith, 2016). PPAR-γ, the most widely investigated subtype, is expressed in the epidermis and the hair follicles (HFs), and controls skin barrier permeability, inhibits keratinocyte proliferation, and promotes epidermal terminal differentiation (Ruzehaji et al., 2015). We have previously shown that agonistic PPAR-γ modulators may exert protective functions on keratin 15+ epithelial progenitor cells in human HFs, while they inhibit hair growth by inducing catagen and inhibiting the proliferation of hair matrix keratinocytes (Ramot et al., 2014).

Recently, PPAR-γ–mediated signaling has also been implicated in the regulation of mitochondrial energy metabolism, that is, in adipose tissue and the brain (Chaturvedi and Flint Beal, 2013; Hock and Kralli, 2009). However, it remains to be studied whether PPAR-γ stimulation impacts on the mitochondrial biology of human HF keratinocytes in situ, which are known to be key players in HF energy metabolism (Klopper et al., 2015). Therefore, we have asked whether the selective (agonistic) PPAR-γ modulator, N-acetyl-GED-0507-34-Levo (N-acetyl-GED) (Ramot et al., 2014), can modulate mitochondrial properties, using microdissected, organ-cultured human scalp HFs as a physiologically and clinically relevant assay system (Langan et al., 2015).

In order to screen whether PPAR-γ stimulation can lead to changes in mitochondria-related genes, we have re-analyzed our previously executed genome-wide microarrays (data are accessible through Gene Expression Omnibus series accession number GSE109009), performed on two independent sets of organ-cultured HFs from a female patient’s scalp, treated with 0.01 mM N-acetyl-GED for 6 hours (Ramot et al., 2014). Using more permissive selection criteria (P < 0.05, >2-fold, equidirectional changes in both patients), four genes involved in the control of mitochondrial function were found to be up-regulated (Supplementary Table S1 online), in line with the hypothesis of a role for PPAR-γ stimulation in mitochondrial function.

To further test the possible role of PPAR-γ in mitochondrial function, human anagen VI HFs were micro-dissected from normal scalp skin that was obtained after written informed consent from two healthy patients, as described previously (Ramot et al., 2011), adhering to Helsinki guidelines, and under a license from the ethics committee of the University of Münster (reference no.: 2015-602-f-S). The HFs were organ-cultured for 6 days with vehicle or 0.01–1 mM N-acetyl-GED (concentrations were chosen based on the previously identified optimal dose [Ramot et al., 2014]), with change of culture media every 48 hours.

To confirm a possible role for N-acetyl-GED in mitochondrial function, we investigated expression of four genes that are known to be key players in mitochondrial biology, namely MTO1, PGCIα, TFAM, and SLC25A3 (Ramot et al., 2011; Vidali et al., 2014). Quantitative RT-PCR analyses revealed that there was a significant and concentration-dependent stimulation of PGCIα transcription, while for the other genes, there was a strong trend toward up-regulation of mRNA levels following the treatment (Figure 1a).

To further dissect the stimulating effect of N-acetyl-GED on key mitochondrial elements, we also analyzed their expression at the protein level. N-acetyl-GED significantly increased immunoreactivity for MTO1, a key enzyme of the respiratory chain (Knuever et al., 2012; Vidali et al., 2016) (Figure 1b). This increase was observed in both the outer root sheath (ORS) and hair matrix keratinocytes. A very similar effect was also evidenced for the protein expression of TFAM, a key transcription factor for mitochondrial DNA synthesis (Knuever et al., 2012; Vidali et al., 2016) (Figure 1c). A slightly different effect was observed when we checked the protein expression of VDAC1, a reliable marker for mitochondrial mass in general (Sorgato and Moran, 1993; Vidali et al., 2014). In the lower concentration (0.01 mM), there was a slight decrease in the protein expression. However, as with the other mitochondrial markers, the two higher concentrations led to significant up-regulation of immunoreactivity.

Abbreviations: HF, hair follicle; N-acetyl-GED, N-acetyl-GED-0507-34-Levo; ORS, outer root sheath; PPAR, peroxisome proliferator–activated receptor

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Figure 1. N-Acetyl-GED modulates mitochondrial activity in microdissected human HFs. (a) mRNA levels of PGC1α, MTCO1, TFAM, and SLC25A3, in human HFs, treated with 0.01 or 0.1 mM N-acetyl-GED for 6 hours. Mean ± SEM. (b) N-acetyl-GED stimulated MTCO1 immunoreactivity after 6 days in culture. Mean ± SEM, n = 11–20 HFs per group. Insets: higher magnification of the MTCO1 staining in the ORS keratinocytes. (c) N-acetyl-GED stimulated TFAM immunoreactivity after 6 days in culture. Mean ± SEM, n = 15–19 HFs per group. (d) While slightly decreasing VDAC1 immunoreactivity in the lower concentration, N-acetyl-GED stimulated VDAC1 immunoreactivity in the higher concentrations after 6 days in culture. Mean ± SEM, n = 7–12 HFs per group. (a–d): *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, as indicated, using two-tailed, unpaired Student t test. DP, dermal papilla; HF, hair follicle; MK, matrix keratinocytes; N-acetyl-GED, N-acetyl-GED-0507-34-Levo; ORS, outer root sheath. Bars = 50 μm.
in both the ORS and matrix keratinocytes (Figure 1d).

As complementary evidence, we also tested the effects of N-acetyl-GED on isolated human ORS keratinocytes. Interestingly, we found that 6-hour treatments by using the same, non-cytotoxic (non-cytotoxicity determined by MTT and CyQUANT assays, Supplementary Figure S1a–S1d online) concentrations (i.e., 0.01, 0.1, and 1 mM) of N-acetyl-GED were able to significantly up-regulate expressions of all the tested “mitochondrion-relevant” genes (i.e., MTCO1, TFAM, PGC1α, VDAC1, and SLC25A3) in the cells of the investigated donor compared to the vehicle-treated group (Figure 2a). Thus, results of this pilot experiment revealing more prominent actions on “pure” ORS keratinocyte cultures than in intact HFs (Figure 1a) invite the hypothesis that, within the HFs, ORS keratinocytes may be the primary targets of N-acetyl-GED-mediated PPAR-γ activation.

Furthermore, to obtain independent, indirect proof of the influence of PPAR-γ stimulation on the mitochondrial actions, we also measured ATP release of ORS keratinocytes upon the above 6-hour N-acetyl-GED treatments (for details, see the Supplementary Materials and Methods online). This showed that N-acetyl-GED concentration-dependently increased the amount of ATP released by cultured human ORS keratinocytes, highlighting again that it is indeed likely to positively regulate mitochondrial activity in these cells (Figure 2b).

N-acetyl-GED has been shown before to induce catagen and decrease hair matrix keratinocyte proliferation (Ramot et al., 2014), while the current study shows that N-acetyl-GED promotes mitochondrial energy metabolism. These findings are in agreement with the concept that the HF mainly engages in aerobic glycolysis and does not rely predominantly on mitochondria-dependent glucose metabolism (Philpott and Kealey, 1990; Williams et al., 1993). These data also raise the question whether the catagen-promoting impact of N-acetyl-GED may be dependent on the promotion of catagen-/terminal differentiation-associated processes in the hair bulb, which are more reliant on oxidative phosphorylation.

These preliminary results suggest that, similar to its effects in other tissues, PPAR-γ-mediated signaling is a player in regulating the energy metabolism of human scalp HFs by enhancing mitochondrial function, most probably primarily in the ORS keratinocytes. Next, it deserves to be evaluated whether this modulation of mitochondrial biology read-out parameters by PPAR-γ modulators in human skin shown here can be translated into clinically beneficial effects (e.g., anti-HF aging).

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PPAR-γ Regulation of Mitochondrial Function in Hair Follicles

**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.01.033.
Vildagliptin Significantly Increases the Risk of Bullous Pemphigoid: A Finnish Nationwide Registry Study


Bullous pemphigoid (BP) is the most common autoimmune blistering skin disease (Schmidt and Zillikens, 2013). BP has become more common over the past two decades (Forsti et al., 2014; Joly et al., 2012; Langan et al., 2008). However, the underlying causes of the increasing incidence of BP are poorly understood. Altogether, over 50 drugs have been reported to induce BP (Stavropoulos et al., 2014). The use of dipeptidyl peptidase-4 inhibitors (DPP-4i), a class of drug used for the treatment of diabetes, has recently been scrutinized as a risk factor for BP, both in case reports (see Supplementary Table S1 online) and in national pharmacovigilance database reports (Bene et al., 2016; Garcia et al., 2016), but large population-based studies are lacking. In this study we investigated the potential association between DPP-4i and BP using data from Finnish national registries.

Populations (Table 1), databases used, and statistical analysis are described in the Supplementary Materials online. After adjusting for diabetes and several neurological disorders, the use of any DPP-4i was associated with a significantly increased risk of BP compared with the control population (Table 2). The use of vildagliptin was associated with 10-fold elevated risk for BP. Combination therapy regimens containing metformin and sitagliptin or vildagliptin were associated with an increased risk of BP, but metformin alone was not associated with a difference in BP risk. A sensitivity analysis supported these findings (Table 2, and see Supplementary Table S2 online). The use of DPP-4i had no significant impact on patient age at BP diagnosis when subjects who had received a DPP-4i were compared with those who had not (77.7 vs. 76.7 years), starting from 2007 when the first DPP-4i was approved in Finland. The mean latency from vildagliptin exposure to BP diagnosis was 449 days (see Supplementary Table S3 online). In women, the risk of having BP diagnosis after DPP-4i medication was heightened compared with men (see Supplementary Table S4 online).

To the best of our knowledge, no previous nationwide registry study has reported an association between vildagliptin and BP. These results concur with previous observations from

Abbreviations: BP, bullous pemphigoid; DPP-4i, dipeptidyl peptidase-4 inhibitor