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Adiponectin promotes hyaluronan synthesis along with increases in *hyaluronan synthase 2* transcripts through an AMP-activated protein kinase/peroxisome proliferator-activated receptor-α-dependent pathway in human dermal fibroblasts

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ABSTRACT

Although adipocytokines affect the functions of skin, little information is available on the effect of adiponectin on the skin. In this study, we investigated the effect of adiponectin on hyaluronan synthesis and its regulatory mechanisms in human dermal fibroblasts. Adiponectin promoted hyaluronan synthesis along with an increase in the mRNA levels of *hyaluronan synthase 2* (*HAS2*), which plays a primary role in hyaluronan synthesis. Adiponectin also increased the phosphorylation of AMP-activated protein kinase (AMPK). A pharmacological activator of AMPK, 5-aminoimidazole-4-carboxamide-1β-ribofuranoside (AICAR), increased mRNA levels of *peroxisome proliferator-activated receptor-α* (*PPARα*), which enhances the expression of *HAS2* mRNA. In addition, AICAR increased the mRNA levels of *HAS2*. Adiponectin-induced *HAS2* mRNA expression was blocked by GW6471, a PPARα antagonist, in a concentration-dependent manner. These results show that adiponectin promotes hyaluronan synthesis along with increases in *HAS2* transcripts through an AMPK/PPARα-dependent pathway in human dermal fibroblasts. Thus, our study suggests that adiponectin may be beneficial for retaining moisture in the skin, anti-inflammatory activity, and the treatment of a variety of cutaneous diseases.

1. Introduction

Adiponectin, an adipocyte-derived hormone, is abundant in the plasma of healthy humans (8.9 ± 5.4 μg/ml) [1]. Low circulating levels of adiponectin in obese or overweight patients are thought to contribute to pathogenesis of the metabolic syndrome and act as risk factors for type 2 diabetes mellitus and cardiovascular disease with abdominal obesity and insulin resistance [2,3]. Adiponectin receptor 1 and 2 (AdipoR1 and AdipoR2) have been discovered as a new class of heptahelix receptors, which are structurally and functionally distinct from G-protein-coupled receptors. Downstream signaling of both AdipoRs is mainly mediated by phosphorylation of AMP-activated protein kinase (AMPK) and activation of peroxisome proliferator-activated receptor-α (PPARα), which regulates cellular metabolic processes including glycolysis, gluconeogenesis, lipid metabolism, and protein synthesis [4]. Several studies have shown that PPARα signaling pathways may act as interesting therapeutic targets for a broad variety of skin disorders, including psoriasis and atopic dermatitis, and skin malignancies [5,6]. Although a number of studies have examined the effects of adiponectin on several types of cells and tissues [7,8], its effects on the extracellular matrix in skin remains unclear.

Hyaluronan is a major extracellular matrix component of the skin and plays important roles in moisturizing properties, formation of scaffolds to promote tissue repair or regeneration, and biological functions such as cellular proliferation and migration [9]. Akazawa et al. [10] recently reported that adiponectin increased the mRNA levels of *hyaluronan synthase 2* (*HAS2*), the major producer of hyaluronan in the dermis, but the precise regulatory mechanisms are still unknown.

In the present study, we used human dermal fibroblasts to examine the effect of adiponectin on hyaluronan synthesis and its regulatory mechanisms related to the AMPK/PPARα pathway.

2. Materials and methods

2.1. Materials

Fetal bovine serum (FBS) was purchased from JRH Biosciences (Lenexa, KS, USA). Recombinant human adiponectin was purchased

Abbreviations: HAS2, *hyaluronan synthase 2*; AMPK, AMP-activated protein kinase; AICAR, 5-aminoimidazole-4-carboxamide-1β-ribofuranoside; PPARα, peroxisome proliferator-activated receptor-α; AdipoR, adiponectin receptor; FBS, fetal bovine serum; MEM, modified Eagle's medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate buffered saline; PBST, PBS containing 0.5% (v/v) Tween 20.

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from BioVision (Mountain View, CA, USA). ProteoExtract Subcellular Proteome Extraction Kit was obtained from Calbiochem (Darmstadt, Germany). Amersham Hybond-P PVDF Membrane, Amersham ECL Advance Western Blotting Detection Kit, and ECL Advance™ blocking agent were obtained from GE Healthcare UK Ltd. (Little Chalfont, England). TaqMan® Gene Expression Cells-to-CT™ Kit, TaqMan universal PCR master mix core reagent kit, and TaqMan Gene Expression Assays kits were obtained from Applied Biosystems (Foster City, IN, USA). GW6471 was purchased from Tocris Cookson Ltd. (Bristol, UK). Anti-AMPKα antibody, anti-phospho-AMPKα (Thr-172) antibody, and 5-aminoimidazole-4-carboxamide-1β-ribofuranoside (AICAR) were obtained from Cell Signaling Technology (Boston, MA, USA). ECL™ anti-rabbit IgG-horseradish peroxidase-linked whole antibody was obtained from Rockland Immunochemicals, Inc. (Gilbertsville, PA, USA). A hyaluronan assay kit was purchased from Seikagaku Biobusiness Co. (Tokyo, Japan). RC DC protein assay was purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA).

2.2. Cell culture and treatment

The human dermal fibroblast cell line Detroit 551 was obtained from the American Type Culture Collection (Manassas, VA, USA), and cultured in modified Eagle's medium (MEM) containing 10% (v/v) FBS, 1% (v/v) MEM non-essential amino acid solution, and 1% (v/v) sodium pyruvate in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3. RNA extraction and quantitative PCR

Human dermal fibroblasts were seeded in 96-well plates in the culture medium at a density of 5×10^3 cells/well and were incubated for 24 h, followed by serum starvation for 24 h. The cells were treated with or without adiponectin (10 μg/ml) for 0, 2, 4, 8, 12, and 24 h. In an additional experiment, the cells were treated for 8 h with AICAR (0, 0.1, or 0.3 mM). For experiments using an inhibitor, cells were treated with adiponectin (0 or 10 μg/ml) in the presence of various concentrations of GW6471 (0, 1, or 3 μM). Following treatment, total RNA was extracted and cDNA was prepared with the TaqMan® Gene Expression Cells-to-CT™ Kit according to the manufacturer's instructions. The amplification products of *HAS2* were detected using TaqMan universal PCR master mix core reagent kit and TaqMan Gene Expression Assays kits. The mRNA level of *HAS2* was measured by quantitative PCR using an ABI Prism 7300 apparatus (Applied Biosystems), and the levels were expressed as values relative to that of *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*). Amplifications were performed under the following conditions: 2 min at 50 °C and 10 min at 95 °C, followed by 50 cycles of 15 s at 95 °C and 1 min at 60 °C.

2.4. Quantification of hyaluronan

The culture supernatant fraction was collected after 0, 2, 4, 8, 12, and 24 h of incubation with or without adiponectin (10 μg/ml). The level of hyaluronan was measured with the hyaluronan assay kit according to the manufacturer's instructions.

2.5. Immunoblot analysis

Human dermal fibroblasts were seeded in 6-well plates at a density of 1×10^5 cells/well. When the cells were confluent, the medium was exchanged for serum-free MEM. Twenty-four hours later, the cells were treated with adiponectin (10 μg/ml) for 0, 5, 15, 30, or 60 min. The cells were lysed with ProteoExtract Subcellular Proteome Extraction Kit according to the manufacturer's instructions. The total amount of protein in the lysate was measured with an RC DC protein assay reagent. Equal amounts of protein lysates were loaded and electrophoresed on a 10% (w/v) Tris–glycine polyacrylamide gel in SDS. Following electrophoresis, the proteins were transferred onto a PVDF membrane using a transfer buffer. The membrane was subsequently blocked with phosphate buffered saline (PBS) containing 2% (w/v) ECL Advance blocking agent and 0.05% (v/v) Tween 20 for 1 h. The membrane was incubated with an anti-phospho-AMPKα (Thr-172) antibody at 4 °C overnight, washed 5 times with PBS containing 0.5% (v/v) Tween 20 (PBST), and incubated with anti-rabbit IgG-horseradish peroxidase (1:2500 dilution in the blocking buffer) for 1 h. After washing the membrane 5 times with PBST, immunoreactive bands were visualized using Amersham ECL Advance Western Blotting Detection Kit. To confirm the phosphorylation status of the AMPKα subunit, reprobing with an anti-AMPKα antibody was performed.

2.6. Statistical analysis

Results are expressed as mean ± SD. Statistical differences between 2 groups were determined using the Student's *t*-test. The mean of multiple groups were compared by the Student–Newman–Keuls test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Adiponectin induced hyaluronan synthesis and the expression of HAS2 mRNA in human dermal fibroblasts

Fig. 1A and B show the time-course effect of adiponectin on hyaluronan synthesis and the expression of *HAS2* mRNA in cultured dermal fibroblasts. The level of hyaluronan secreted into the medium increased in a time-dependent manner following the addition of adiponectin (10 μg/ml), and significantly increased after 24 h in comparison to the cells without adiponectin. The *HAS2* mRNA expression was significantly upregulated and subsequently reached a peak at 8 h after the addition of adiponectin (10 μg/ml).

3.2. Effect of adiponectin on AMPK activation in human dermal fibroblasts

To investigate the effect of adiponectin on AMPK activity, the phosphorylation status of the AMPKα subunit in human dermal fibroblast was determined after the treatment of adiponectin (10 μg/ml) for 0, 5, 15, 30, or 60 min (Fig. 2). The results showed that treatment with adiponectin induced Thr-172 phosphorylation of AMPKα.

3.3. Effect of AICAR, a pharmacological activator of AMPK, on gene expression of PPARα and HAS2 in human dermal fibroblasts

Treatment with AICAR significantly increased the expression level of *PPARα*, a downstream target of AMPK (Fig. 3A). Likewise, AICAR treatment significantly enhanced the level of *HAS2* mRNA in a dose-dependent manner (Fig. 3B).

3.4. GW6471, a PPARα antagonist, inhibited adiponectin-induced HAS2 mRNA expression

As shown in Fig. 4, the adiponectin-induced *HAS2* mRNA expression was blocked by GW6471 in a concentration-dependent manner. GW6471 alone had no effect on the basal expression level of *HAS2* mRNA.