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지방유래줄기세포의 지방분화과정에서 활성산소가 미치는 영향

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Role of Reactive Oxygen Species in the Adipogenesis of Adipose-derived Stem Cells

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Purpose: Stem cells continue to receive research attention in the clinical fields, and adipose-derived stem cells (ADSCs) have been shown to be a good source raw material. Many plastic surgeons are researching the ADSC adipogenesis with a view of conducting clinical trials, and many attempts have been made to identify the factors that promote the adipogenesis of ADSCs, but comparatively few correlation studies have been undertaken to explore the relation between reactive oxygen species (ROS) and the ADSC adipogenesis. We undertook this study is to investigate the effects of ROS on ADSC adipogenesis.

Methods: ADSCs were isolated and cultured from abdominal adipose tissue, and cultured in different media; 1) DMEM (control), 2) adipogenesis induction culture medium, 3) adipogenesis induction culture medium with ROS (20 μM/50 μM H_2O_2), 4) adipogenesis induction culture medium containing ROS (20 μM/50 μM H_2O_2) and antioxidant (10 μM/ 20 μM Deferoxamine). We compared adipogenesis in these different media by taking absorbance measurements after Oil-Red O staining every 5 days.

Results: After culturing for 20 days, significant differences were observed between these various culture groups. Absorbance results showed significantly more adipogenesis had occurred in media containing adipogenesis induction culture medium and H_2O_2 (in a H_2O_2 dose-dependently manner) than in media containing adipogenesis induction culture medium and no H_2O_2 (p< 0.001). Furthermore, in media containing adipogenesis induction culture medium, H_2O_2, and antioxidant, absorbance results were significantly lower than in adipogenesis induction culture medium and H_2O_2 (p<0.001).

Conclusion: These findings suggest that ROS promote the adipogenesis of ADSCs. We suggests that ROS could be used in the adipose tissue engineering to improve fat cell differentiation and implantable fat tissue organization.

Key Words: Reactive oxygen species (ROS), Adipose-derived stem cells (ADSCs), Adipogenesis

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I. INTRODUCTION

Stem cell studies continue to be actively performed in various scientific and clinical fields. In particular, tissue engineering based on stem cells has received much attention in the plastic surgery field, and today adipose-derived stem cells (ADSCs) are considered a suitable raw material for tissue engineering. Furthermore, adipose tissue is a readily available source of ADSCs, which have multi-potent ability, and can be differentiated into osteoblasts, chondrocytes, endothelial cells, myoblasts, and other cell types.[1,2]

Many plastic surgeons hope to be able to use adipose tissues differentiated from ADSCs in soft tissue reconstructions, and in animal studies, such tissues have been produced using different scaffolds and successfully implanted.[3-5]

Many studies involving inhibitors and promoters of adipocyte maturation have been performed to elucidate the adipogenesis pathway and to promote successful implantation. Today adipose tissue is attracting research attention, because of the increased focus on metabolic diseases, such as, obesity and type 2 diabetes.[6,7] Furthermore, free radical formation and oxidative stress have

been shown to be related to metabolic diseases.[8,9] Consequently, researchers have started investigating the effects of reactive oxygen species (ROS) on the adipogenesis of ADSCs,[4,10-12] but the nature of the relation between ROS and adipogenesis has yet to be elucidated.

The aim of this study is to evaluate the effects of ROS on the adipogenesis of ADSCs by examining the differentiation of ADSCs into adipocytes in the presence of ROS (hydrogen peroxide, H_2O_2).

II. MATERIALS AND METHODS

ADSCs preparation

ADSCs were separated from human adipose tissues in remnant TRAM (Transverse Rectus Abdominis Musculocutaneous) flaps after breast reconstructive surgery. Washed and chopped adipose tissues were digested with 0.5% type I collagenase (Worthington, Lakewood, NJ, USA) for 1 hour at 37℃. Tissues were then filtered, washed, and centrifuged. Floating mature adipocytes were discarded, ADSCs were obtained from precipitated pellets.

ADSCs were seeded in dishes and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS (fetal bovine serum) (Invitrogen-Gibco, Grand Island, NY, USA) and A/A (antibiotics/antimycotics) (Welgene Inc., Daegu, Korea) under normoxic conditions at 37℃.

Adipogenesis of ADSCs

Cultured ADSCs were plated in 24 well-plates (3×10^4 cells/well) and cultured in the following culture media; 1) DMEM as control, 2) adipogenesis induction culture medium. This was composed of 1 μM dexamethasone, 10 μg insulin, 0.2 mm indomethacin, and 0.5 mm IBMX (3-isobutyl-1-methylxanthine); all purchased from Sigma (St. Louis, MO, USA), 3) adipogenesis induction culture medium containing 20 μM H_2O_2 (Sungkwang Co., Ltd., Bucheon, Korea), 4) adipogenesis induction culture medium containing 50 μM H_2O_2, 5) adipogenesis induction culture medium containing 20 μM H_2O_2 and antioxidant; 10 μM Deferoxamine (Sigma, St. Louis, MO, USA), 6) adipogenesis induction culture medium containing 20 μM H_2O_2 and 20 μM Deferoxamine, 7) adipogenesis induction culture medium containing 50 μM H_2O_2 and 10 μM Deferoxamine, and finally 8) adipogenesis induction culture medium containing 50 μM H_2O_2 and 20 μM Deferoxamine. The concentrations of H_2O_2 and Deferoxamine were determined by several pilot studies.

Adipogenesis quantification

On adipogenesis induction days 5, 10, 15, and 20, cells from each of the above 8 groups were stained with Oil-Red O stain and absorbance was measured using a VERSA max microplate reader (Molecular Devices Ltd., Sunnyvale, CA, USA).

Statistical analysis

Absorbance measurements were analyzed by ANOVA with post-hoc comparison. Statistical significance was accepted for p values of < 0.05.

III. RESULTS

Linear increases in absorbance results were observed in the groups containing adipogenesis induction culture medium or adipogenesis induction culture medium + H_2O_2. On culture days 5, 10, and 15, absorbance results were non-significantly higher in the groups containing adipogenesis induction culture medium + H_2O_2 than in the adipogenesis induction culture medium group. On day 20, absorbance results were significantly higher in the groups containing adipogenesis induction culture medium + H_2O_2 than in the adipogenesis induction culture medium group (p<0.001), and significantly higher in the adipogenesis induction culture medium + 50 μM H_2O_2 group than in the adipogenesis induction culture medium + 20 μM H_2O_2 group (p<0.001).

On culture days 5 and 10, absorbance results were higher in the groups containing adipogenesis induction culture medium + H_2O_2 + Deferoxamine than in the groups containing adipogenesis induction culture medium + H_2O_2. On culture day 15, absorbance results were non-significantly lower in the groups containing adipogenesis induction culture medium + H_2O_2 + Deferoxamine than in the groups containing adipogenesis induction culture medium + H_2O_2. On day 20, absorbance results were significant lower in the groups containing adipogenesis induction culture medium + H_2O_2 + Deferoxamine than in the groups containing adipogenesis induction culture medium + H_2O_2 (p<0.001), but no significant difference

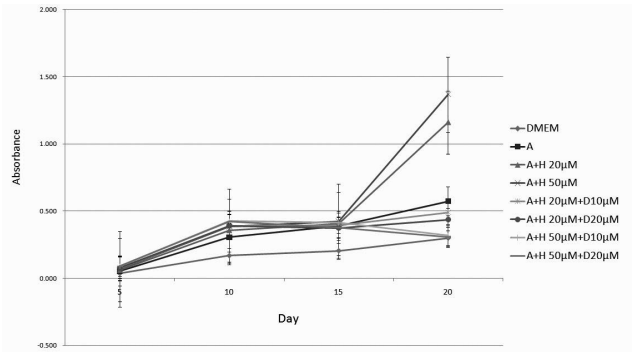

Fig. 1. Adipogenesis of ADSCs was quantified by measuring absorbance after Oil-Red O staining (A; adipogenesis induction culture medium, H; H_2O_2, D; Deferoxamine).

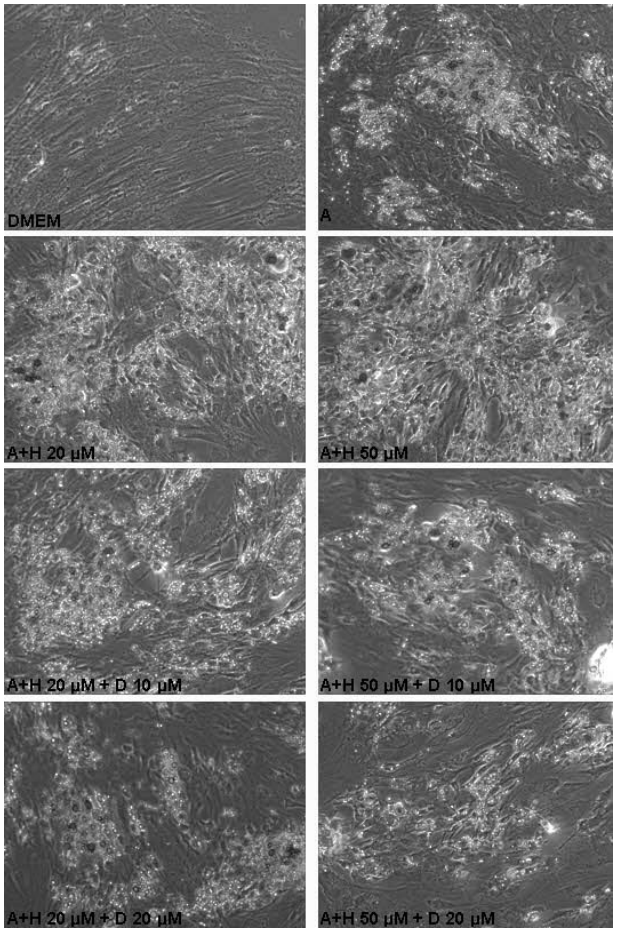

Fig. 2. Adipogenesis of ADSCs was demonstrated by Oil-Red O staining (× 100) (A; adipogenesis induction culture medium, H; H_2O_2, D; Deferoxamine).

was observed between the adipogenesis induction culture medium + H_2O_2 (20 μM/50 μM) + Deferoxamine (10 μM) groups and the adipogenesis induction culture medium + H_2O_2 (20 μM/50 μM) + Deferoxamine (20 μM) groups (Fig. 1, 2).

IV. DISCUSSION

ROS are essentially required by biological systems but they can also have injurious effects. ROS are involved in host defense, cellular signaling systems, and in the induction of mitogenic responses, but ROS excesses and antioxidant deficiencies are harmful to cellular lipids, proteins, and DNA. Furthermore, ROS are known to be actively involved in many human diseases and in the aging process.[13] Today, relations between metabolic diseases, like obesity and type 2 diabetes, and oxidative stress are being actively researched. However, few studies have been conducted on the effect of ROS on ADSC adipogenesis.

The purpose of this study is to determine the effect of ROS on the adipogenesis of ADSCs. The present study shows that ROS dose-dependently promote the adipogenesis of ADSCs. This effect was confirmed by the inhibitory effect of the antioxidant. These findings contrast to those of Pessler-Cohen et al.,[12] who found that free radical formation inhibits adipocyte differentiation by inducing adipocyte insulin resistance. Furthermore, Hwang et al[10] reported that genistein, EGCG (Epigallocatechin gallate), and capsaicin inhibit adipocyte differentiation by activating AMP-activated protein kinase (AMPK). In addition, they demonstrated that ROS is required for the AMPK activation needed to inhibit adipocyte differentiation.

However, clinical studies have shown that increased systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome.[8,9] SchrOder et al.[11] reported that insulin increases ROS production, which mediated by Nox4, and demonstrated that insulin-induced preadipocyte differentiation depends on ROS. These authors stimulated 3T3-L1 cells and human preadipocytes with H_2O_2 in the absence of insulin, and their results showed that preadipocyte differentiation is promoted by H_2O_2. Furthermore, these results coincide well with those of the present study. Aguiari et al.[4] demonstrated that ADSCs and MDSCs (muscle-derived stem cells) differentiate into adipocytes when exposed to high glucose levels, and found that ROS are produced in response to high glucose by stimulating PKCβ. They

also suggested that ROS play an important role during the adipogenesis. Furthermore, in an *in vivo* study, they successfully implanted differentiated adipocytes into nude rats.

The role of oxygen as a signaling molecule for the differentiation of stem cells have become a major research focus. In particular, several *in vitro* studies have shown that the differentiation of several stem cells is enhanced under hypoxic conditions.[14] Hypoxia induces ROS production from the mitochondria.[15] Therefore, ROS may play a role in the mechanism of the effect of hypoxic conditions on ADSCs.

The mechanism of the ROS effect on adipogenesis is uncertain and is certainly worthy of further study. The effects of ROS can be influenced by various factors; ROS concentration, exposure time and pattern of ROS, antioxidant concentrations, and co-exposure of other factors.

V. CONCLUSION

The present study shows that ROS promote the adipogenesis of ADSCs, which suggests that ROS could be used in the adipose tissue engineering field to improve fat cell differentiation and implantable fat tissue organization. Also, our results also support the link between oxidative stress and metabolic diseases. However, much more work needs to be done to determine the nature of the mechanism responsible for the effect of ROS on adipogenesis.

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