Hyaluronic Acid Decreases Lipid Synthesis in Sebaceous Glands

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Hyaluronic acid (HA) is the major glycosaminoglycan in the extracellular matrix and has been implicated in several functions in skin cells. However, evidence is lacking regarding the HA signaling in sebaceous glands, and its potential role needs to be clarified. We investigated the role of HA in lipid production in sebaceous glands in an experimental study of human sebocytes followed by a clinical study. We first examined the effects of HA on sebaceous glands in hamsters and intradermal injection of HA into hamster auricles decreased both the size of sebaceous glands and the level of lipid production. We demonstrated that human skin sebaceous glands in vivo and sebocytes in vitro express CD44 (HA binding receptor) and that HA downregulates lipid synthesis in a dose-dependent manner. To evaluate the clinical relevance of HA in human skin, 20 oily participants were included in a double-blind, placebo-controlled, split-face study, and the HA-treated side showed a significant decrease in sebum production. The results of this study indicate that HA plays a functional role in human sebaceous gland biology and HA signaling is an effective candidate in the management of disorders in which sebum production is increased.

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INTRODUCTION

Hyaluronic acid (hyaluronan, HA) is a glycosaminoglycan composed of glucuronic acid and *N*-acetyl-glucosamine. HA is a major component of the extracellular matrix in skin tissues and is involved in a wide range of cellular functions (Tammi et al., 2002; Vigetti et al., 2014). These findings suggest that in addition to its critical role as a structural component of the extracellular matrix, HA plays pivotal roles in tissue metabolism.

HA acts as an important signal in normal skin physiology and functions. HA induces keratinocyte proliferation, differentiation, cell adhesion, and permeability barrier homeostasis (Bourguignon, 2014; Bourguignon et al., 2006). HA also stimulates fibroblast proliferation and collagen production and has been incorporated into decellularized scaffolds along with basic fibroblast growth factor (Greco et al., 1998). HA may also play a key role in the sequential phases of tissue injury and repair (Noble, 2002) and could contribute to skin aging via age-related decreases in HA production (Tzellos et al., 2009). These findings indicate that there are many possible medical applications of HA for treating a variety of epidermal dysfunctions and damage as well as aging-related skin conditions and wound healing (Bourguignon, 2014; Neuman et al., 2015).

The sebaceous gland plays a vital role in skin homeostasis by producing sebum. However, excessive sebum production induces the formation of primary lesions associated with acne (Zouboulis, 2004). A previous report suggested that the sebaceous gland expresses both HA and its receptor, CD44 (Wang et al., 1992), and suggested a role of HA in sebum production. However, no reports have shown that HA signaling directly influences lipid production. The potential roles and mechanisms of action of HA in human sebaceous glands should also be clarified. In this study, we investigated the regulation of HA on sebaceous lipogenesis in vivo and in vitro. Our findings provide compelling evidence that HA plays significant roles in human sebocyte biology.

RESULTS

HA reduces the size of sebaceous glands and lipid production in the hamster auricle

We first examined the effects of HA on sebaceous glands in hamsters, which are equipped with a pilosebaceous unit that can be compared anatomically and functionally with human sebaceous follicles (Iinuma et al., 2009; Luderschmidt et al., 1983). When auricles were injected with 1 mg/ml HA, the relative size of the sebaceous glands was smaller than that of auricles treated with phosphate buffered saline (PBS) as a control (Figure 1a and c). In addition, Oil Red O staining showed that lipid accumulation was attenuated in sebaceous glands of the HA-treated auricles (Figure 1b), and the skin surface showed a lower sebum level in the HA-injected side than in the control (Figure 1d).

CD44 is presented in human sebaceous glands and primary sebocytes

As a preliminary study, we examined the expression of CD44, which is a functionally important surface receptor

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Abbreviations: Akt, acutely transforming retrovirus AKT8 in rodent T-cell lymphoma; HA, hyaluronic acid; PBS, phosphate buffered saline; siRNA, small-interfering RNA; TLC, thin-layer chromatography

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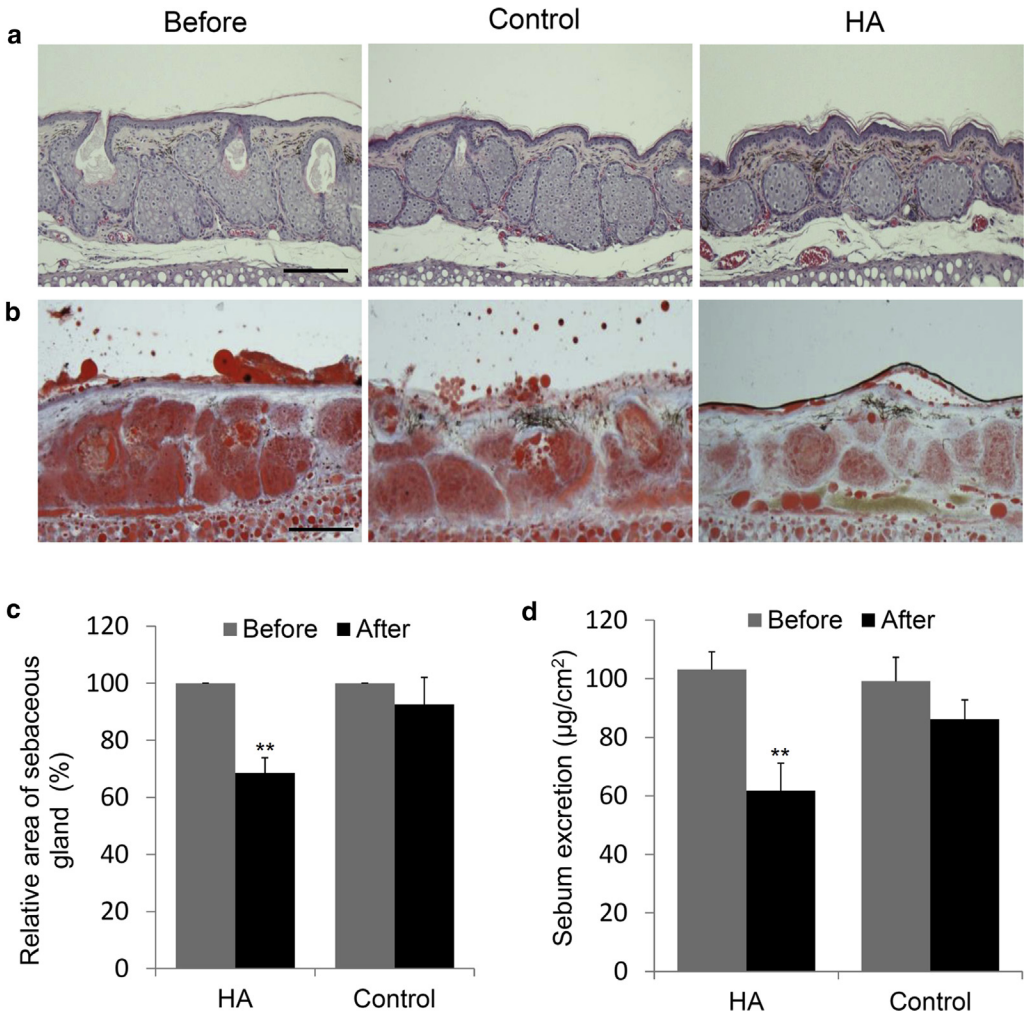

Figure 1. Effects of HA on hamster sebaceous glands. Different groups of six hamster auricles were injected with 1 mg/ml HA or PBS as a control. (**a**) Hematoxylin-eosin and (**b**) Oil Red O staining were performed on frozen tissue sections of the ear 7 days after injection. (**c**) After hematoxylin-eosin staining, the sebaceous gland size was determined in 30 consecutive sebaceous glands. (**d**) Changes in the mean sebum output level (μg/cm^2) after HA injection into the auricles of hamsters. Scale bars = 20 mm. Data represent mean ± SEM (n = 6). Data were analyzed using Student's *t* test (**$P < 0.01$). HA, hyaluronic acid; PBS, phosphate buffered saline; SEM, standard error of the mean.

for HA binding (Underhill, 1992). Immunohistochemical finding demonstrated the presence of CD44 in normal human sebaceous glands (Figure 2a). We then examined the expression of CD44 in human primary sebocytes. The results showed that that the CD44 was also expressed in human sebocytes at the protein and mRNA levels (Figure 2b–d). As a positive control, we used the normal human keratinocytes and fibroblasts, which have been reported to express the CD44 (Pasonen-Seppänen et al., 2012; Wang et al., 1992; Yevdokimova and Podpryatov, 2005), whereas human hepatoma HepG2 cells, which lack CD44 expression, served as a negative control (Olaku et al., 2011). These results suggested a possible role of HA in human sebocyte biology.

HA inhibits proliferation of human sebocytes

Primary sebocytes were treated with HA to explore the potential role of HA in vitro. We first examined the effect of HA on the viability of sebocytes by lactate dehydrogenase release assay. HA at a dose of 100 μg/ml exerted cytostatic activity on cultured sebocytes (Figure 3a). We next investigated the effects of HA on proliferation of sebocytes by [^{3}H]-thymidine incorporation assay, and the results indicated significant suppression of proliferation at 100 μg/ml HA (Figure 3b). In the low cell seeding assays, HA also suppressed the growth rate of human sebocytes at 10 and 100 μg/ml (Figure 3c). These observations suggest that HA efficiently inhibits the proliferation of human sebocytes in vitro.

HA attenuates lipid production in human sebocytes

To evaluate the effects of HA on lipogenesis in human sebocytes, we investigated the lipid droplet in cytoplasm after treating with various doses of HA in differentiated human sebocytes. Previous studies indicated that confluent sebocytes contained larger numbers of intracellular lipid droplets than differentiated sebocytes (Li et al., 2013; Lo Celso et al., 2008). Therefore, we used postconfluent sebocytes to investigate the effects of HA on lipid suppression. As a result, we found that HA decreased intracellular lipid levels in a dose-dependent manner as determined by measuring the optical density after Oil Red O staining (Figure 4a). In addition, when differentiated human sebocytes were treated with various doses of HA, lipid droplets in cytoplasm was reduced on microscopy after staining with Oil Red O or Nile red (Figure 4b). We used thin-layer chromatography (TLC) followed by densitometric analyses to further explore the relative abundance of sebaceous lipid classes. The results of TLC analysis indicated that differentiated human sebocytes