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Original Article

Androgens modulate keratinocyte differentiation indirectly through enhancing growth factor production from dermal fibroblasts

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ABSTRACT

Background: The main pathogenesis of acne vulgaris is increase in sebum production and abnormal keratinization of the hair infundibulum. The androgens are involved in acne pathogenesis by modulating sebaceous glands to enhance sebum production. However, the molecular mechanisms of abnormal keratinization of the hair infundibulum are not fully elucidated.
Objective: We hypothesized that the androgens affect the dermal fibroblasts, another androgen receptor-positive cells in the skin, resulting in abnormal keratinization through keratinocyte-fibroblast interaction.
Methods: We investigated effects of androgens and estrogens on growth factors expressions by RT-PCR and western blot analysis in human fibroblast (hFB), human keratinocyte (hKC), and fibroblast-keratinocyte co-culture. In vivo, we examined the growth factor expression in acne lesions compared to normal hair follicles by laser-assisted confocal microscope.
Results: In vitro, androgens but not estrogens significantly increased amphiregulin (AREG), epiregulin (EREG), fibroblast growth factor (FGF) 10, and insulin-like growth factor binding protein (IGFBP) 5 mRNA and protein expressions in human fibroblasts but not in keratinocytes. In vivo, AREG, EREG, FGF10, and IGFBP5 were more abundant in acne lesion compared to normal facial skin. FGF10 suppressed cytokeratin 1 and cytokeratin 10 expression in hKC, which was along with the decreased ratio of cytokeratin 10 against cytokeratin 14 in acne lesions compared to normal facial skin. Also, DHT suppressed cytokeratin 1 and cytokeratin 10, in fibroblast-keratinocyte co-culture similarly to the effect of FGF10 to hKC.
Conclusion: These observations suggested that androgens enhance growth factors production from dermal fibroblasts, and growth factors from fibroblasts alter keratinocyte differentiation in acne lesion.

1. Introduction

Acne vulgaris (AV) is one of the most common inflammatory skin diseases in dermatological practice. The pathogenesis is multifactorial, and major factors contributing in AV formation are sebum overproduction, *Cutibacterium acnes* (formerly *Propionibacterium acnes*) overgrowth, abnormal inflammation and alteration in follicular keratinization known as comedogenesis [1]. Since the onset of AV usually starts from adolescents for both genders and extends to adult in especially in female [2], the sex hormones, mainly androgens, have been recognized to play roles in acne pathogenesis [3]. Although most of AV patients have normal endocrinological status, the association between AV and androgens was literally reported [4,5]. Also, the effectiveness of anti-androgenic treatments in AV partially proves this concept [6]. Exacerbation of acne in polycystic ovary syndrome is another example of sex hormone involvement in AV [7–9].

Androgens affect the skin via androgen receptors (AR). Sebocytes express functional AR, and androgens enhance sebaceous activity [10]. Other cells in the skin, for example, fibroblasts, and endothelial cells, also express functional AR [10]. However, AR expression in keratinocytes is still controversial. While reports showed positive AR by immunohistochemistry [11,12], the

Abbreviations: AV, acne vulgaris; AREG, amphiregulin; AR, androgen receptors; CK, cytokeratin; Ea, alpha-estradiol; Eb, beta-estradiol; EREG, epiregulin; DHT, dihydrotestosterone; FBS, fetal bovine serum; FGF10, fibroblast growth factor 10; FGFR2, fibroblast growth factor receptor 2; FTM, flutamide; hFB, human fibroblast; hKC, human keratinocyte; IF, immunofluorescent; IGFBP5, insulin-like growth factor binding protein 5; T, testosterone.

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functional AR were not demonstrated by transcriptional activity study [13]. Thus, the involvement of androgens in keratinocyte comedogenesis remains not fully elucidated.

In this study, we hypothesized that the androgens affect keratinocytes proliferation and differentiation indirectly by activation of juxtaposed fibroblasts expressing functional AR. We aimed to determine the influence of sex hormones androgens and estradiols on growth factor production of fibroblasts. We also examined the effect of growth factors produced by fibroblasts on keratinocyte proliferation and differentiation by co-culture of fibroblasts and keratinocytes.

2. Material and methods

2.1. Reagents

Testosterone (T, Cat #; T1500), alpha-estradiol (Ea, Cat #; E8750), beta-estradiol (Eb, Cat #; E2758), and flutamide (FTM, Cat #; F9397) were purchased from Sigma-Aldrich (St Louis, MO, USA) and were dissolved in 99.5% ethanol (Wako Pure Chemical Industries, Japan). Dihydrotestosterone (DHT, Cat #; A0426) was purchased from Tokyo Chemical Industry (Tokyo, Japan) and was dissolved into dimethyl sulfoxide (Wako Pure Chemical Industries, Ltd., Japan). Human Epiregulin (hEREG, Cat #; SRP3033) was purchased from Sigma-Aldrich. Recombinant human Insulin-like Growth Factor Binding Protein-5 (rhIGFBP-5) and recombinant human Fibroblast Growth Factor-10 (rhFGF-10) were purchased from R&D Systems (MN, USA).

2.2. Cell culture

Normal human dermal fibroblasts (hFB, Cat #; KF-4009) and normal human epidermal keratinocytes (hKC, Cat #; KK-4009) were purchased from Kurabo Industries (Japan). The passage numbers of hFB and hKC were between 2–12 and 2–4, respectively. hFB were cultured in Medium 199 without phenol red (Cat #; 11043-023, Gibco, Thermo Fisher Scientific, Waltham, Massachusetts, USA) supplemented with 10% of Fetal Bovine Serum (FBS). hKC were cultured in Humedia-KG2 with supplements (Cat #; KK-2150S, Kurabo, Japan). For studies in monoculture of hFB or hKC, 500,000 cells of hFB or hKC were cultured in a 6-cm tissue culture dish (Falcon®, New York, USA). On the next day, the cultured media were replaced with the medium without FBS for hFB cultures nor supplements for hKC cultures. On the third day, T, DHT, Ea, Eb, and FTM were added without changing the media. In some experiments, hKC were stimulated with 1 and 10 ng/ml of hEREG, rhFGF10, and rhIGFBP5 for 24 h and were harvested for RNA and protein analysis.

In co-culture experiments, 150,000 cells per a well of hFB were cultured in 6-well plates until 80% confluency was reached. Then 450,000 cells per a well of hKC were introduced on the hFB layer, and hFB and hKC were co-cultured in a mixture of Medium 199 and Humedia-KG2 with supplements in 1:1 ratio. After 24 h co-culturing, we changed mixed media without supplements and stimulated with the hormones. RNA was collected from the co-cultures at 48 h after the hormone stimulation.

All experiments were performed with triplicate samples in each stimulus and repeated at least thrice to confirm the reproducibility.

2.3. RNA isolation, reverse transcription, quantitative real-time PCR

Total RNA was collected and isolated by Isogen (Cat #; 311-02501, Nippon Gene, Japan) as previously described [14,15]. The amount and quality of RNA were measured by Nanodrop One/One^c Microvolume UV–vis Spectrophotometer (Thermo Fisher Scientific). cDNA was made from 1 μg of total RNA by reverse-transcription with PrimeScript™ RT-PCR Kit from Takara Bio (Japan). Quantitative real-time PCR was performed by Stratagene Mx3000 P RoHS (Agilent Technologies) with Brilliant II Ultra-Fast QPCR Master Mix protocol (Agilent Technologies) and Taqman® Gene Expression Assays listed in Supplementary Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The amount of transcripts was normalized to GAPDH using ΔΔCT method.

2.4. Western blotting

Total protein was collected into RIPA buffer (Cat #; 89900, Thermo Fisher Scientific). Total Protein quantity was measured by BCA protein assay (Thermo Fisher Scientific). The detailed protocol for western blot were previously described [16]. Briefly, SDS sample buffer (Cell Signaling Technology) was added to lysates and heated at 99 °C for 5 min. The lysates were electrophoretically separated in 12% polyacrylamide gel (ATTO Corporation, Tokyo, Japan) and then electrically transferred into polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). At room temperature for an hour, the 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween-20 (TBST) was used as the blocking agent. After three times washing by TBST, the membranes were incubated overnight at 4 °C with the primary antibodies listed in Supplementary Table 2. On the second day, the membranes were washed several times with TBST and incubated with secondary antibodies: horseradish peroxidase (HRP)-conjugated goat anti-rabbit anti IgG or HRP-conjugated goat anti-mouse IgG antibodies (Cell Signaling Technology). By using 20X LumiGlo (Cell Signaling Technology), the stained proteins were identified by Image Quant Software (Molecular Dynamics, Sunnyvale, CA). β-actin was used as an internal control.

2.5. Immunofluorescent (IF) staining

Paraffin-embedded blocks with acne vulgaris lesions from four male patients and uninvolved region of four surgically excised melanocytic nevus samples in facial skin (normal skin samples) were recruited. Three μm-thick sections were placed onto the glass slides. After deparaffinized with serial concentrations of xylene and ethanol, the slides were washed several times with PBS containing 0.1% Tween-20 (PBST). The PBST with 3% normal goat serum was used as blocking agent for 30 min. The slides were incubated with primary antibodies listed in Supplementary Table 2 for overnight at 4 °C. After several wash with PBST, the slides were incubated with secondary antibodies purchased from Abcam: Alexa Flour®568 goat anti-mouse IgG (H + L), Alexa Flour®568 goat anti-rabbit IgG (H + L), or Alexa Flour®488 goat anti-mouse IgG (H + L). Nuclei were stained by DAPI-Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL, USA). The IF-stained sections were visualized and digitally recorded by LSM700 laser scanning confocal microscope with the ZEN 2011 software (Carl Zeiss, Thornwood, NY, USA). The thickness of cytokeratin 14 (CK14)- and CK10-positive layers in epidermis at hair infundibular area and interfollicular epidermis were measured by ZEN2011 software. In each specimen, the areas near the hair infundibular area and interfollicular epidermis were chosen to measure. The lines were drawn perpendicularly from the dermo-epidermal junction to the surface of epidermis. The graphs were plotted according to the intensity of the immunofluorescence and the point of differentiation was defined as the intersection of the graph representing the CK14 and CK10 positive layers. The data were obtained from 3 AV and 3 normal skin samples, and the mean ± standard deviation (SD) of CK10/CK14 ratio were calculated and statistically analyzed. The protocol of the study was approved by the Ethical Committee of the Tohoku University Graduate School of Medicine.

2.6. Cell viability assay

After 24 h of hormone incubation, hFB and hKC culture media were replaced by media with 10% alamarBlue® Cell Viability Reagent (Thermo Fisher Scientific), then the cultures were incubated at 37 °C for 4 h. OD560 and 600 nm were measured using the SpectraMAX microplate reader, and cell viability was calculated to normalized with control.

2.7. Statistical analysis

One-way ANOVA with multiple comparison technique was performed by GraphPad Prism7 (GraphPad Software, La Jolla, CA, USA). All experiments were examined in triplicate fashion. Unless otherwise specified, the data in the figures was presented in mean ± SEM. The significant level was set as $P < 0.05$. Statistically significant differences compared to control were represented by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

3. Results

3.1. Androgens increased expression of AREG, EREG, FGF10, and IGFBP5 in fibroblasts, but not in keratinocytes

To examine the effect of androgens and estradiols on fibroblasts, we stimulated hFB with Testosterone (T), Dihydrotestosterone (DHT), alpha-Estradiol (Ea), and beta-Estradiol (Eb) at 10^{-7} and 10^{-6} M for 24 h and examined expression of growth factors. We observed significant increase in AREG, EREG, FGF10, and IGFBP5 mRNA expression by T and DHT (Fig. 1a). The increase was approximately 3–4 folds compared to control in AREG, EREG, and FGF10. While, Ea and Eb did not show significant increase of these mRNA expressions. In the western blot studies, DHT increased the protein expression of AREG, ERG, FGF10, and IGFBP5 parallelly to mRNA expression (Fig. 1b). The increase was highest at 24 h by DHT stimulation.

We next examined if the hormones affect growth factor expression in keratinocytes. In contrast to the effects of androgens on fibroblast, we did not observe the significant changes of mRNA expressions of AREG and EREG in hKC by the androgens and estradiols (Fig. 1c). FGF10 and IGFBP5 gene were not detectable in hKC from our RT-PCR procedures. The hormones also did not affect the expression of cytokeratins CK1, CK10, CK5, and CK14 in hKC. (Fig. 1d). To examine if the androgens and estradiols affect cell viability/proliferation, we examined hFB and hKC viability by the alamarBlue® Cell Viability Assay. We did not observe significant differences of cell viability between the hormone-treated cells and non-treated cells (Supplementary Fig. 1). Thus, we concluded that androgens can increase mRNA and protein expressions of growth factors AREG, EREG, IGFBP5, and FGF10 in fibroblasts, but affect little on keratinocyte dynamics.

3.2. Acne lesions showed higher expression of AREG, EREG, FGF10 and IGFBP5 than normal skin

According to our in vitro study results, we examined AREG, EREG, FGF10, and IGFBP5 expressions in AV lesions and normal human skin by IF staining. We observed the markedly higher intensity of AREG, EREG, FGF10, and IGFBP5 stains in AV lesions compared to normal skin (Fig. 2). With higher magnification, we also noted that increased stain signals were predominantly located in the dermis close to hair infundibulum. These observations *in vivo* supported our hypothesis that AREG, EREG, FGF10 and IGFBP5 from fibroblasts involve in AV pathogenesis.

3.3. Altered CK10/CK14 ratio in hair infundibulum of acne compared to normal hair follicles

We paid attention to cytological patterns of keratinocytes in the hair infundibular areas where the acne comedogenesis is thought to be originated. By using CK14 and CK10 stains as markers of undifferentiated and differentiated keratinocytes, respectively, we measured the thickness and calculated the ratio of stratum basale and suprabasal layers of epidermis by using the laser scanning confocal microscopy with the software ZEN 2011 (Fig. 3a). We noticed that the CK10/CK14 ratio was significantly lower in AV lesions compared to controls (Fig. 3b, c, f). To eliminate the possibility of different epidermal thickness from tangential slides during process, we also measured the epidermal thickness at hair follicle and CK10/CK14 ratio from epidermis in interfollicular areas, and observed no significant differences for both measurements (Fig. 3d, e, f). These suggested altered processes in keratinocyte differentiation in hair infundibula of AV.

3.4. FGF10 and DHT-treated fibroblasts suppressed CK1 and CK10 mRNA expression in keratinocytes

To explain the altered keratinocyte differentiation observed in AV lesions, we conducted two sets of *in vitro* experiments to examine keratinocyte differentiation; effects of growth factors in keratinocyte monoculture and effects of sex hormones in co-culture of fibroblasts and keratinocytes. We stimulated hKC with FGF10 for 24 and 48 h and observed that CK1 and CK10 mRNAs were significantly suppressed by FGF10 at both 1 and 10 ng/ml concentration compared to controls (Fig. 4a). FGF10 also suppressed CK5 mRNA at 48 h. FGF10 slightly but not significantly suppressed CK14 mRNA at 48 h. EREG 10 ng/ml significantly increased CK1 mRNA at 24 h but decreased CK5 mRNA at 48 h compared to control though the differences between the control were less than 2-folds (Supplementary Fig. 3a). IGFBP5 10 ng/ml significantly but less than 2-folds increased CK1 and CK10 mRNA compared to control at 48 h (Supplementary Fig. 3b). FGF10, EREG and IGFBP5 did not affected the keratinocyte cell viability (Supplementary Fig. 2).

We next examined the cytokeratin expression in keratinocytes that were co-cultured with fibroblasts and stimulated with 10^{-7} M of T, DHT, Ea, and Eb for 48 h. DHT suppressed CK1 and CK10 mRNA expression in keratinocytes co-cultured with fibroblasts (Fig. 4b). CK5 and CK14 were not significantly changed. Since DHT did not alter cytokeratin expression in monoculture of keratinocytes (Fig. 1d), these findings suggested the action of DHT to keratinocyte differentiation was took place indirectly via effect of DHT on hFBs.

3.5. The androgen receptor antagonist flutamide suppressed the growth factor mRNA expressions in androgen-treated fibroblasts

To explore the involvement of androgen receptor in growth factor induction, we stimulated hFB with 10^{-7} and 10^{-6} M of the androgen receptor antagonist flutamide (FTM) for 24 h and examined expression of growth factors. FTM is a non-steroidal selective antagonist of AR without other hormonal activities [17]. FTM alone at both 10^{-7} and 10^{-6} M did not significantly alter AREG, EREG, FGF10, and IGFBP5 mRNA expression compared to control (Fig. 5a). Next, we examined the effect of FTM on androgen-induced growth factor mRNA expressions in fibroblasts. FTM partially suppressed the expression of growth factors stimulated by T (Fig. 5b). However, FTM did not affect the DHT-induced AREG, EREG, and FGF10 expressions in hFB.

4. Discussion

In the series of in vitro and in vivo observations in this article, we propose that the androgens alter the keratinocyte

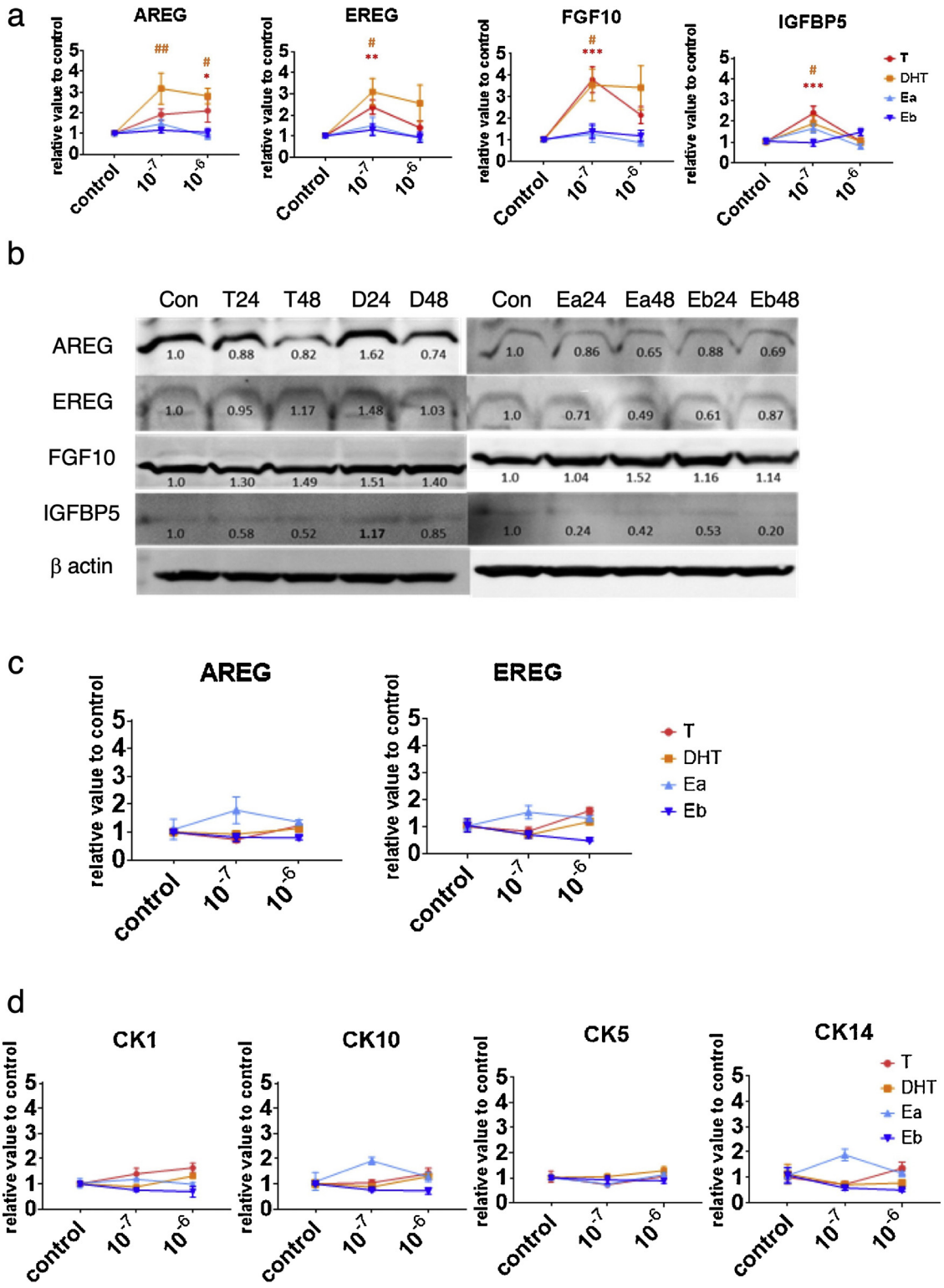


Fig. 1. Androgens induced growth factors in human fibroblasts but not in human keratinocytes. (a, b) Normal human fibroblasts were stimulated with 10^{-7} or 10^{-6} M of testosterone (T), dihydrotestosterone (DHT), estrogen-alpha (Ea) or estrogen-beta (Eb) for 24 h, and amphiregulin (AREG), epiregulin (EREG), fibroblast growth factor 10 (FGF10) and insulin-like Growth Factor Binding Protein 5 (IGFBP5) mRNA expressions (a) and protein expressions (b) were examined. (c, d) Normal human keratinocytes were stimulated with 10^{-7} or 10^{-6} M of T, DHT, Ea or Eb for 24 h, and AREG and EREG mRNA expressions (c) and cytokeratin CK1, CK10, CK5 and CK14 mRNA expressions (d) were examined. The data was shown as mean ± SEM of relative values compared to control. T, **Red** lines; DHT, **orange** lines; Ea, light **blue** lines; Eb, **blue** lines. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in T. # $P < 0.05$, ## $P < 0.01$ in DHT. (N = 3).

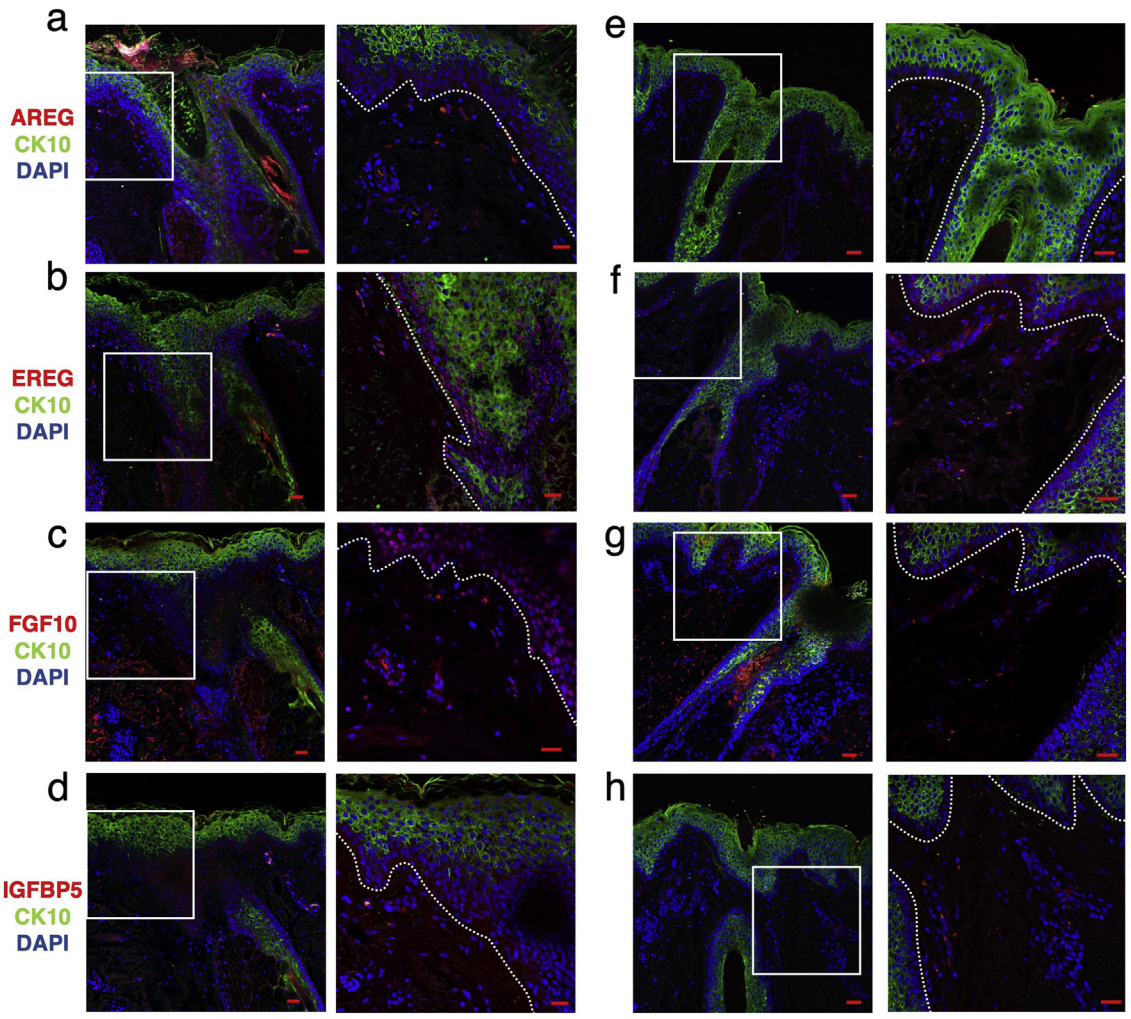
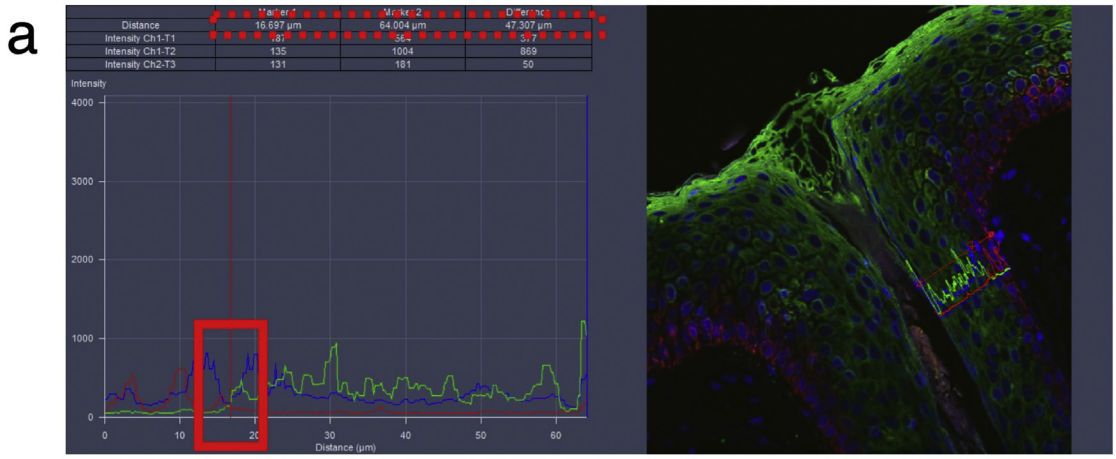


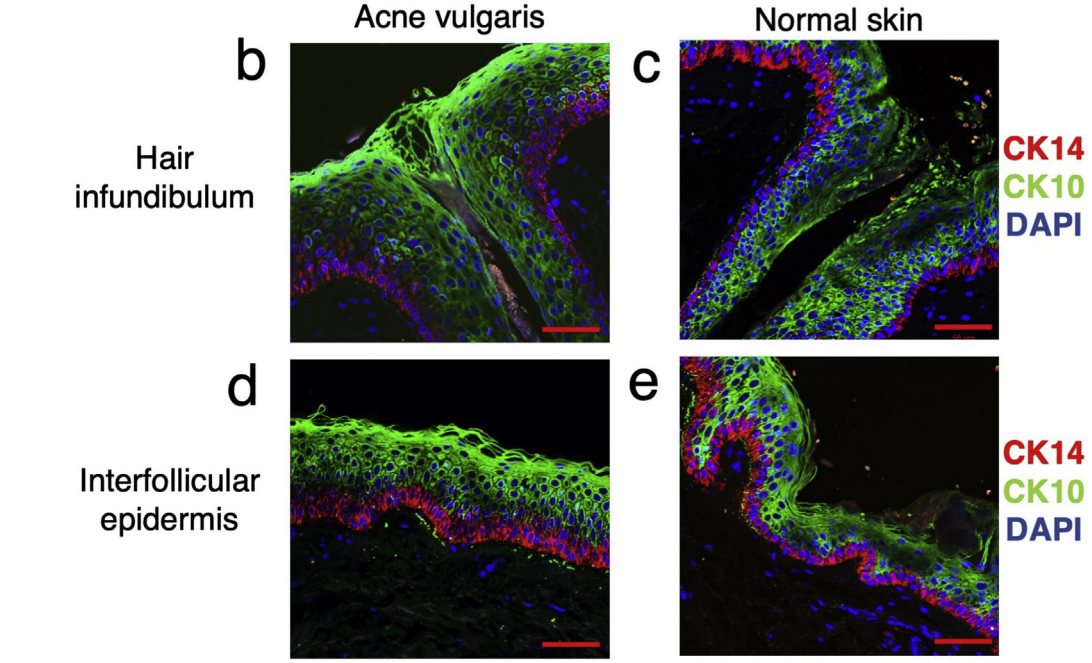
Fig. 2. Growth factor expressions in the dermis of acne lesions. The expressions of amphiregulin (AREG), epiregulin (EREG), fibroblast growth factor 10 (FGF10) and insulin-like Growth Factor Binding Protein 5 (IGFBP5) in acne vulgaris lesions (a, b, c, d) and normal hair follicles (e, f, g, h) were examined by immunofluorescent stains and visualized in **red** by laser-assisted confocal microscope. Cytokeratin 10 (CK10) are showed in **green.** The higher magnified images of indicated areas are presented on the right. Scale bars = 20 μm.

differentiation through the growth factor production from fibroblasts (Fig. 6). Dermal fibroblasts, stimulated by androgens via androgen receptors (ARs), can secrete FGF10 and other growth factors. Subsequently, FGF10 alters keratinocyte differentiation as shown by altered cytokeratin expression in vitro experiment. These in vitro observations are in accordance with the expression of FGF 10 in dermis and altered cytokeratin ratio in hair infundibulum in AV lesions. This would explain another mechanism of comedogenesis in AV.

The follicular hyperkeratinization is one of the main factors contributing in the comedogenesis of AV [1]; however, the exact mechanism of abnormal keratinization has not been clearly elucidated. The changes of keratinocytes located in infrainfundibular area, where is considered as the origin of comedo formation, are still controversial. Using CK6, CK16, CK17, and Ki-67 tissue staining, several studies supported the hyperproliferation and abnormal differentiation of infundibular keratinocytes [18–20]. In contrast, recent studies declined the hyperproliferative evidences in AV [21,22]. Currently, it is believed that inflammation triggers the process and results in abnormal keratinization via IL-1α [23,24]. Also, androgens, especially DHT, indirectly involve in this process by causing abnormal sebum content produced by sebaceous glands where ARs are abundant [25]. In our skin, ARs are not only expressed on sebocytes, but also on dermal fibroblasts. Our results demonstrated that DHT may indirectly suppress keratinocyte differentiation, but not proliferation, via the effect of FGF10 produced by dermal fibroblasts and that there was a decreased CK10/CK14 ratio in IF-stained sections at hair infundibulum of AV compared to normal hair follicles. Combining observations in previous publication and our data in the article, these findings suggest androgens are involved in the abnormal differentiation process of AV hair infundibulum, which is crucial in acne pathogenesis.

FGF10 is highly homologous to FGF7 and known as keratinocyte growth factor 2. Its function is mediated via FGFR2b expressed on keratinocytes [26]. Fibroblasts, stimulated by T and DHT, express more FGF7 and FGF10. Through the mitogen-activated protein kinase (MAPK)-, phospholipase C-γ/protein kinase (PKC)-, and phosphoinositide-3-kinase (PI3K)/Akt pathways, receptor dimerization activates downstream cascades causing cell cycle transcription factor activation in keratinocytes [26]. Abnormal function of FGF7/10 to FGFR2 pathway causing abnormal keratinocyte proliferation and differentiation is found in Apert syndrome, and AV is one of main clinical symptoms of Apert syndrome [27]. Previous studies examined the effect of FGF10 in hKC monocultures incubated in heparin- and Ca^{2+}-added media for six days and proposed that the FGF10 can exert keratinocyte proliferation and promote early (CK1 and CK10) and late keratinocyte





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Area	Acne vulgaris	Normal skin	*P* value
CK10/CK14 ratio (Mean ± SD)	Hair Infundibulum	2.38 ± 0.27	4.56 ± 0.97	0.041*
	Interfollicular epidermis	1.53 ± 0.14	2.07 ± 0.32	0.15
Epidermal thickness (µm) (Mean ± SD)	Hair follicle	94.59 ± 8.62	75.53 ± 6.73	0.11

Fig. 3. Altered expression of cytokeratins in AV hair infundibulum. (a) The thickness of cytokeratin 14 (CK14, **red)** and cytokeratin 10 (CK10, **green)**-positive layers were measured from the dermo-epidermal junction by using ZEN2011 software. The solid **red** box in the graph represents the border of the CK14 **(Red** color/lines) and CK10 **(Green** color/lines) where the keratinocyte differentiation took place. The distances from the dermo-epidermal junction to the border and to the surface of epidermis were shown in the dotted red box above. The ratio of the differentiated keratinocytes layer versus basal layer of epidermis in the area was calculated by the ratio of the distance of the **green** curve divided by the red curve (CK10/CK14 ratio). (b–e) Representative images of CK14 **(red)** and CK10 **(green)**-positive layers in hair infundibular area and interfollicular epidermis in acne vulgaris and normal skin are shown. Scale bars are 50 µm. (f) The thickness of epidermis was measured at 6 different areas from each section and the ratio of CK10/CK14-positive layer thickness was calculated. The mean ± SD are presented. * $P < 0.05$. (N = 3).

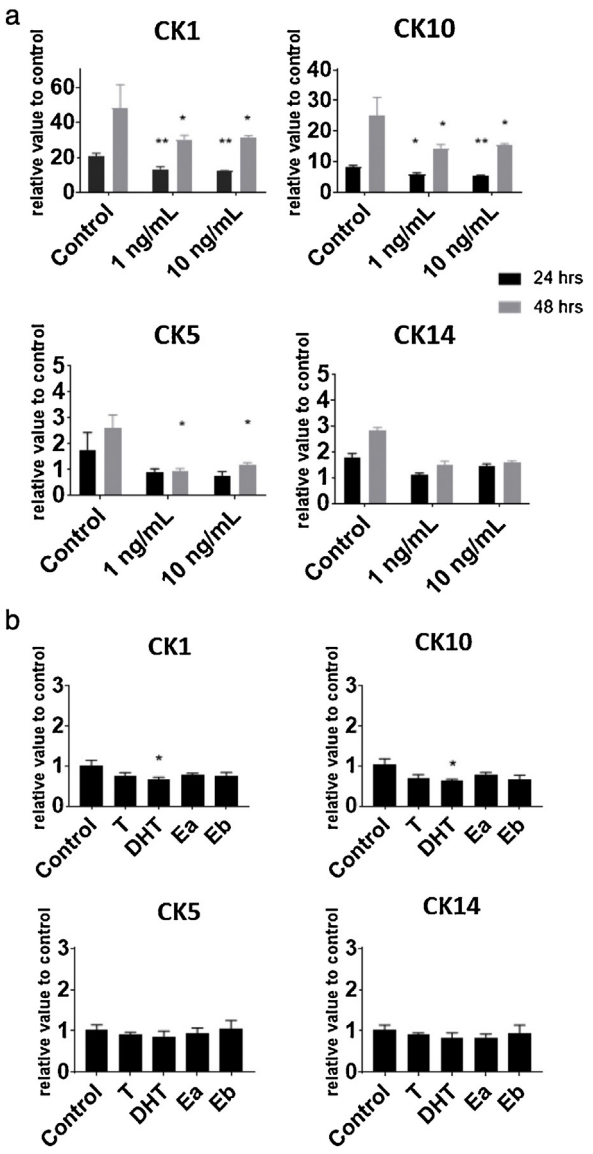

Fig. 4. FGF10 and DHT-treated fibroblasts altered cytokeratin mRNA expression in keratinocytes. (a) Human keratinocytes were stimulated with 1 or 10 ng/ml of FGF10, and cytokeratin 1 (CK1), cytokeratin 5 (CK5), cytokeratin 10 (CK10) and cytokeratin 14 (CK14) mRNA expressions were measured at 24 and 48 h. (b) Co-culture of hFB and hKC were stimulated with testosterone (T), dihydrotestosterone (DHT), estrogen-alpha (Ea) or estrogen-beta (Eb) for 48 h, and cytokeratin expression were examined. The data was shown as mean ± SEM of relative values compared to control at 0 h (not shown in the graphs). * $P < 0.05$, ** $P < 0.01$. (N = 3).

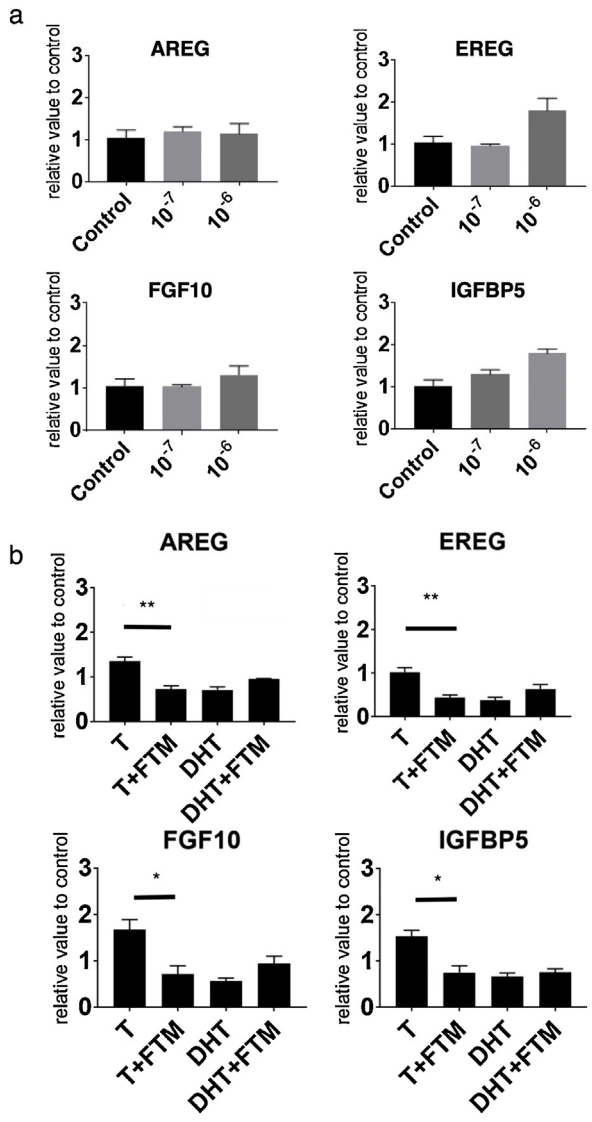

Fig. 5. Androgen receptor antagonist partially suppressed androgen-induced growth factors in human fibroblasts. (a) Normal human fibroblasts were stimulated with 10^{-7} and 10^{-6} M of flutamide (FTM) for 24 h, and amphiregulin (AREG), epiregulin (EREG), fibroblast growth factor 10 (FGF10) and insulin-like Growth Factor Binding Protein 5 (IGFBP5) mRNA expressions were examined. (b) Normal human fibroblasts were stimulated with 10^{-7} M of T and DHT concurrently with 10^{-6} M of FTM for 24 h, and AREG, EREG, FGF10, and IGFBP5 mRNA expressions were examined. The data was shown as mean ± SEM of relative values compared to non-stimulated control (not shown in the graphs). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (N = 3).

differentiation markers (filaggrin) [28,29]. However, the figure in their western blot studies showed that CK10 protein expression by FGF10 seemed to be slightly lower than control and there were no reports regarding CK5 and CK14 changes. In contrast, our study using hKC monoculture with media without supplements (Ca^{2+} and heparin) showed that FGF10 suppressed CK1, CK10, CK5, and CK14 mRNA expression but not affected the keratinocyte proliferation. The discrepancies may be a result from the different incubation period, the additive mitogenic effect of heparin to FGF10 on hKC [30] and the different way of sample analysis. Similarly, FGF7 suppressed of keratinocyte differentiation (CK10 and transglutaminase) in skin equivalent model system [31]. FGF10 null mice showing thinner epidermis and less granular layers than control mice support our findings [32]. Conditional FGF10-Tg mice induced papilloma with epidermal hyperplasia in skin [33]. These observations confirmed that FGF7/10 alter the status of keratinocyte differentiation.

AR has been identified in many cells of the skin, namely, fibroblasts, endothelial cells, smooth muscle cells, sebocytes and also keratinocytes [10,34]. However, there are controversial reports in AR expression on hKCs; positive immunostaining in hKCs [11,12] but no functional mRNA detected [13]. Our in vitro study showed no valid CT values of AR mRNA during RT-PCR analysis (data not shown). At least in the series of our experiments, we believe that there is no functional ARs on hKCs. In the contrast, AR expression in human fibroblasts is not questionable. Androgens, together with their coregulators, affect in fibroblast function in several mechanisms via AR through both genomic and non-genomic pathways [35,36]. In this study, we demonstrated the fibroblast-keratinocyte interaction under influence of androgens.

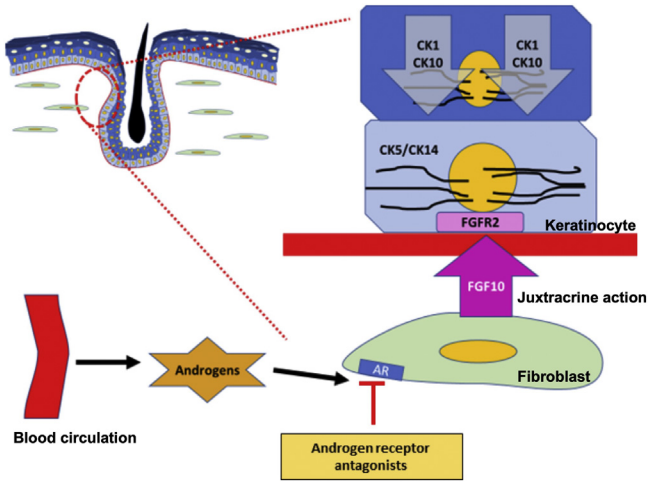

Fig. 6. The proposed pathophysiology of abnormal keratinization by androgens in acne lesions. Androgens from the blood circulation stimulate dermal fibroblasts to produce FGF10 via androgen receptors. FGF10, which binds to FGFR2 on adjacent basal keratinocytes, alters keratinocyte differentiation resulting in CK1 and CK10 suppression in the suprabasal layers and altered CK10/CK14 ratio in hair infundibulum of acne lesions. Androgen receptor antagonists partially suppress the effect of androgen. AR, androgen receptor; CK1, cytokeratin 1; CK5, cytokeratin 5; CK10, cytokeratin 10; CK14, cytokeratin 14; FGF10, fibroblast growth factor 10; FGFR2, fibroblast growth factor receptor 2.

Similarly to our results, epithelial-stromal fibroblast interaction was shown in prostate as androgens promote the epithelial proliferation via stromal fibroblasts [37]. Our study demonstrated that androgens modified keratinocyte differentiation via fibroblast-mediated growth factor production in co-culture of keratinocytes and fibroblasts. Both our study and previous observations in prostate suggest androgens influences stroma-epithelial biology through the juxtracrine manners.

Flutamide (FTM) is a non-steroidal selective antagonist of AR without other hormonal activities [17]. We used FTM in this study because FTM is approved to use for prostate cancer in human and is occasionally used for acne treatments [6,38]. In this study, FTM suppressed of in T-induced FGF10 expression in hFB. Although the effect of FTM was not so dramatic in vitro, the similar action of FTM can be expected in human therapy. Because, FTM turns more active form by hydroxylation in vivo [39], and AR binding activities of FTM is lower compared to other AR blockers in vitro assay [40]. Thus, the inhibition of AR would modulate keratinocyte dynamics through attenuation of growth factor production from hFB.

In conclusion, our data suggest that androgen indirectly modify keratinocytes differentiation through production of growth factors and cytokines from neighboring dermal fibroblasts. This machinery would be in a part of pathogenesis of comedogenesis in acne.

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Conflicts of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.01.007.

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