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treatment, hundreds of new lesions arose on the trunk. Due to this vast increase, laser therapy was discontinued.
In all cases, the treatment was well tolerated under topical anesthesia with lidocaine 25 mg/g + prilocaine 25 mg/g cream (EMLA cream, AstraZeneca, London, UK). The patients reported wound healing within one week.
Ablative lasers are regularly used in the treatment of benign dermal tumours [3-6]. Several authors describe a drilling technique, where the handpiece is held at the exact same place for a longer period of time in order to treat deep seated tumours, while minimizing the epidermal defect [7, 8]. This drilling technique lacks standardization and the results depend largely on the physician's surgical skills. Motion artefacts and variable ablation depths will result in different ablation craters and possibly scarring. In contrast, the use of timed exposure settings enables the creation of reproducible and narrow columns of ablation at a predefined depth. Large numbers of tumours may be removed in one session. However, as our third patient shows, determining the right indication for treatment at timed exposure settings remains an important issue. Treatment efficacy is limited in tumours that are larger than the laser spot or in rapidly erupting lesions, such as eruptive vellus hair cysts [9]. Tumours exhibiting great variation in size impose practical difficulties, since laser settings have to be adjusted many times. In conclusion, our findings indicate that timed exposure CO_2 laser drilling can be a fast and effective treatment for some benign dermal tumours, with a low risk of persistent side effects. However, its value in the treatment of larger and rapidly erupting tumours is limited. ■

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Advanced glycation end-products inhibit mesenchymal-epidermal interaction by up-regulating proinflammatory cytokines in hair follicles

Advanced glycation end-products (AGEs) increase with age in most organs and tissues and they are involved in the onset of aging-related diseases by damaging cellular functions [1, 2]. In the cutaneous field, it has been reported that AGEs induce apoptosis and cellular senescence in the skin [3]. However, despite the fact that some types of alopecia are closely related with age, whether or not AGEs could trigger these hair problems remains unknown.
Dermal papilla cells (DPCs) play essential roles in hair follicular homeostasis by interacting with other cells, such as hair matrix cells [4]. Disturbances in the mesenchymal-epidermal interaction may cause hair abnormalities. In androgenetic alopecia, DPCs up-regulate several cytokines, including IL-6 and TGF-β. These cytokines inhibit the proliferation of hair matrix cells [5, 6]. In our current work, we investigated how AGEs are involved in the intercellular communication between mesenchymal and epidermal cells.
As shown in *figure 1A*, AGEs were observed in the dermal papilla of a mouse skin section. Therefore, it is possible that the AGEs affect the function of dermal papilla cells. DPC-conditioned media (DCM) were prepared and introduced into cultures of human primary keratinocytes (KCs). The proliferation rate of KCs was significantly suppressed when DCM was prepared in the presence of AGE-BSA *(figure 1B)*. Since AGE-BSA did not directly affect the proliferation rates of KCs and DPCs, respectively (data not shown), it is likely that AGE-BSA up-regulates inhibitory molecules in DPCs. Accordingly, in order to understand the molecular basis of the inhibitory action of AGE-BSA, the expression levels of the possible target genes were investigated by semi-quantitative RT-PCR. When DPCs were incubated with AGE-BSA, the alopecia-related pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8 and TNF-α) were dramatically up-regulated in a dose-dependent manner *(figure 1C)*. Transient up-regulation of TNF-α was observed with a peak at 3 hours and expressions of the other cytokines reached peaks at 6 hours (data not shown). Moreover, increased protein levels of IL-6 and TNF-α were observed with the AGE treatment *(figure 1D)*. These results are in good agreement with previous reports in various mammalian cells [1].

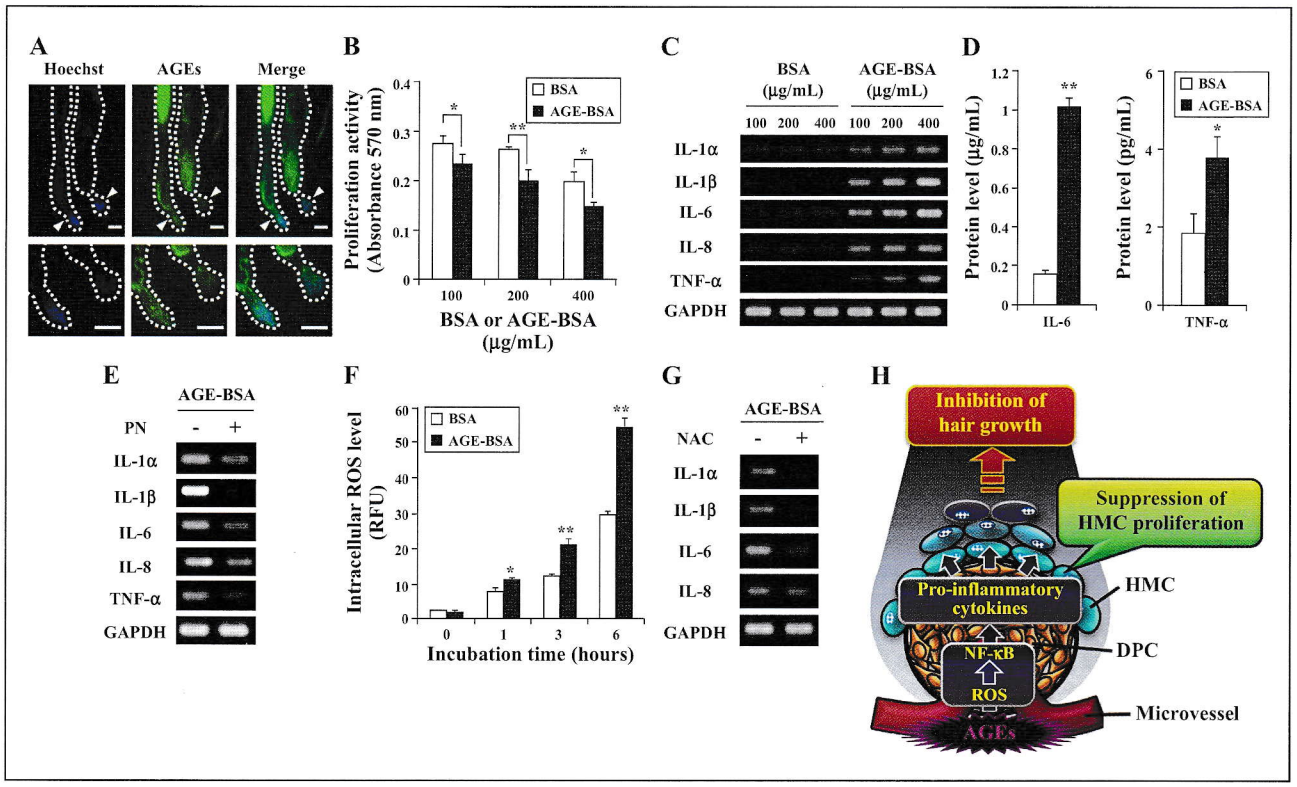

Figure 1. Effects of AGE-BSA on the interaction between DPCs and KCs. **A)** Hair follicular localization of AGEs. Hair follicles are indicated by dotted lines. Arrowhead, dermal papilla. Scale bar, 20 μm. **B)** Effects of AGE-BSA on the proliferation activities of KCs. KCs were cultured with DPC-conditioned medium (DCM) in the presence of 100-400 μg/mL BSA or AGE-BSA for 3 days. The proliferation activities were determined by cell viability assays. Data are represented as the mean ± SD of the three independent experiments. Statistical analysis was performed using Student's *t*-test. *, $p<0.05$; **, $p<0.005$. **C)** Dose-dependent up-regulation of the cytokines by AGE-BSA. DPCs were treated with 100-400 μg/mL BSA or AGE-BSA for 6 hours. **D)** Increased protein levels of IL-6 and TNF-α with AGE-BSA treatment. DPCs were incubated with 400 μg/mL BSA or AGE-BSA for 3 days. The protein levels of IL-6 and TNF-α were measured by ELISA. Data are represented as the mean ± SD of three independent experiments. Statistical analysis was performed using Student's *t*-test. *, $p<0.05$; **, $p<0.005$. **E)** Inhibitory effects of a NF-κB inhibitor (parthenolide, PN) on the expressions of AGE-BSA-induced proinflammatory cytokines. DPCs were pre-incubated with 5 μM parthenolide for 90 minutes followed by treatment with 400 μg/mL AGE-BSA for 6 hours. **F)** AGE-BSA increases intracellular ROS. DPCs were treated with 400 μg/mL BSA or AGE-BSA for 1, 3 and 6 hours after DCFDA treatment. Data are represented as the mean ± SD of three independent experiments. Statistical analysis was performed using Student's *t*-test. *, $p<0.05$; **, $p<0.005$. **G)** Suppressive effects of a ROS inhibitor (*N*-acetyl-*L*-cysteine, NAC) on the expressions of the proinflammatory cytokines induced by AGE-BSA. DPCs were pre-treated with 25 mM NAC for 90 minutes before stimulation by 400 μg/mL AGE-BSA for 6 hours. **H)** A model of AGE-related alopecia. AGEs induce alopecia-related pro-inflammatory cytokines via a ROS-mediated NF-κB pathway, which suppresses the proliferation of hair matrix cells (HMCs).

The effects of parthenolide (an NF-κB inhibitor) and *N*-acetyl-*L*-cysteine (a ROS scavenger) on the expression of pro-inflammatory cytokines were examined for a better understanding of the underlying signaling mechanism. The NF-κB inhibitor remarkably suppressed the AGE-induced pro-inflammatory cytokines *(figure 1E),* which suggests that AGEs up-regulate those pro-inflammatory cytokines via an NF-κB pathway in DPCs. Furthermore, AGE-BSA increased intracellular ROS and the ROS scavenger inhibited the AGE-induced expression levels of IL-1α, IL-1β, IL-6 and IL-8 *(figures 1F,G).* Therefore, we concluded that AGEs induce pro-inflammatory cytokines, mainly via the ROS-mediated NF-κB pathway in DPCs. Further details of the AGE-related pathway will be discussed elsewhere.

In our current model, upon interaction with DPCs, the accumulated AGEs caused an up-regulation of various pro-inflammatory cytokines through the ROS-mediated NF-κB pathway. These cytokines accordingly suppressed hair growth by inhibiting the proliferation of hair matrix cells *(figure 1H).*

In the case of androgenetic alopecia, DPC-derived IL-6 inhibits the elongation of the human hair shaft by suppressing the proliferation of hair matrix cells [6]. Therefore, we speculate that AGEs accelerate the onset of androgenetic alopecia by up-regulating inflammatory cytokines. On the other hand, senescent alopecia develops after 50-60 years of age and it is characterized by diffuse hair thinning over the whole scalp. A previous study showed that the gene expression of NF-κB was enhanced in the scalp of senescent alopecia [7]. Our finding implies that AGEs trigger the senescence of hair follicles and progress age-related alopecia by inhibiting mesenchymal-epidermal interaction. Hair follicular AGEs will be a therapeutic target for the prevention of the age-related hair problems. ■

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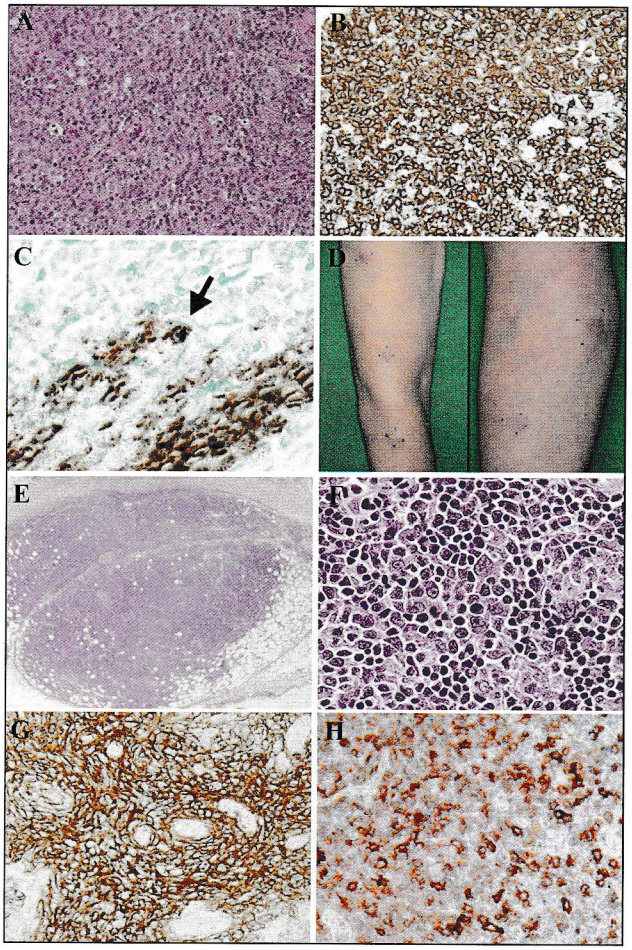

Figure 1. (A) Histological examination of lymph nodes. Numerous, medium-to-large sized, atypical, mononuclear cells with irregular nuclei (Hematoxylin–eosin, ×400). **(B)** Increasing number of CD21$^+$ follicular dendritic cells around high endothelial venules (×400). **(C)** *In situ* hybridization for Epstein-Barr virus (EBV)-encoded RNA (EBER), followed by the immunostaining of CD20. EBER$^+$ cells were positive for CD20 (indicated by arrow) (×800). **(D)** Multiple subcutaneous nodules in her bilateral upper extremities. **(E, F)** Histopathological examination of the nodule on the arm. Diffuse infiltration of medium-to-large sized, atypical lymphocytes in the subcutaneous tissues (Hematoxylin–eosin, **(E)** ×100, **(F)** ×800). **(G, H)** Atypical lymphocytes were positive for CD20 **(G)** and CD30 **(H)** (×400).

Methotrexate-associated lymphoproliferative disorder: Sequential development of angioimmunoblastic T-cell lymphoma-like lymphoproliferation in the lymph nodes and diffuse large B-cell lymphoma in the skin in the same patient

A 66-year old Japanese woman was diagnosed with polymyalgia rheumatica in October 2012 and was treated with methotrexate (MTX) at a dose of 10mg/week. Swelling of the inguinal lymph nodes appeared in February 2013. Histological examination of the lymph nodes revealed numerous, medium-to-large, atypical mononuclear cells with irregular nuclei (*figure 1A*). Increased numbers of CD21$^+$ follicular dendritic cells were observed around the high endothelial venules (*figure 1B*). The neoplastic cells stained positively for CD3 and CD5 but not for CD20 and bcl-6. Epstein-Barr virus (EBV)-encoded RNA (EBER) positive cells by *in situ* hybridization were also positive for CD20 and CD79α, but not CD3 (*figure 1C*). Quantitative polymerase chain reaction for plasma EBV DNA showed 290 copies/10^6 cells (normal: <20). The diagnosis of MTX-associated angioimmunoblastic T-cell lymphoma (AITL)-like lymphoproliferative disorders (LPD) was established and MTX was withdrawn in February 2013. Immunosuppressive agents, such as cyclosporine, tacrolimus and corticosteroids, were not used after the withdrawal of MTX. Swelling of the lymph nodes completely disappeared 6 months after the discontinuation of MTX. In April 2014, she noticed multiple 2-to-3 cm subcutaneous nodules in her bilateral upper extremities (*figure 1D*). Abnormal laboratory results included only elevated soluble interleukin 2 receptor (1291 U/ml,

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