LB1711

The transfection of double-stranded DNA induces cell senescence in cultured human keratinocytes

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As the accumulation of senescent cells in tissues contributes to the impairment of their homeostasis and increases the risk of many age-related diseases, cellular senescence has become a research focus. Since Hayflick reported irreversible cell-cycle arrest in diploid cells, several senescent cell biomarkers, including enlarged and flattened cell morphology, senescence-associated secretory phenotype (SASP) such as IL-6 and IL-8, senescence-associated β-galactosidase (SA-β-Gal) activity and γ-H2AX, have been accepted. In addition, cytosolic double-stranded DNA (dsDNA) was recently identified as a novel senescence marker in mouse embryonic fibroblasts. In skin, although studies on cell senescence in cultured fibroblast are conducted flourishingly, there are few studies on keratinocyte senescence in the culture. Therefore, we characterized keratinocyte senescence induced by transfection of dsDNA, comparing with the senescence depending on replicative stress (cell passaging). The dsDNA-transfected normal human epidermal keratinocytes (NHEKs) demonstrated enlarged and flattened shape, similar to passage 5 (P5) NHEKs. The mRNA expression levels of IL-6 and IL-8 in dsDNA-transfected NHEKs were significantly upregulated to 4.01±0.24 and 3.62±0.54 fold respectively as well as the levels were significantly enhanced in NHEKs after P4. The expression of senescent-associated SA-β-Gal activity was identified in the dsDNA-transfected P4 NHEKs, while SA-β-Gal activity was detected in P5 NHEKs. In addition, ds-DNA transfection accelerated obvious γ-H2AX expression in P4 NHEKs, whereas the expression was detected in P5 NHEKs. Conclusively, these results suggested that dsDNA transfection into NHEKs induced cell earlier than cell passaging-induced senescence, suggesting that dsDNA transfection into the cells provides an *in vitro*-model for studies on cellular senescence.

LB1712

A clinical study of atomic-force microscopy-based rapid atopic dermatitis severity assessment

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This study introduces high-speed dermal atomic force microscopy (HS-DAFM) as a diagnostic technique to assess the severity of atopic dermatitis (AD). The study enrolled five AD patients and a control group of similarly aged individuals with no history of skin barrier disease. Corneocytes were collected using the tape stripping method and imaged using a HS-DAFM. A machine learning algorithm was developed to analyze the images and identify circular nano-objects (CNOs) on the corneocyte surface. The CNO count per unit area was used to assess the severity of AD. To validate HS-DAFM as medical diagnostic device, its output was compared with traditional clinical severity assessments. The study found that the HS-DAFM images from the control group displayed normal skin nanotexture (low CNO count), while the images of the lesional area from AD patients displayed significant morphological changes (high CNO count), with a large surface coverage and mean CNO counts ranging from 270 to 550. A CNO count above 200 was associated with clinical AD symptoms. The two subjects with the most severe AD exceeded a CNO count of 400. Both the number and area of CNOs increased in skin closer to the lesional area. The p-values of "control vs. AD non-lesional" and "control vs. AD lesional" were 0.0030 and 0.0098, respectively. HS-DAFM produced results in seconds, 50 times faster than traditional AFM, and orders of magnitude faster than biochemical assays. These findings suggest that HS-DAFM is an effective tool for rapid AD severity assessment, enabling preclinical treatment, quantitative evaluation of efficacy, and informing self-care to prevent AD symptom progression. These capabilities allow dermatologists to manage AD more effectively, potentially improving the quality of life for millions of AD patients worldwide.

LB1713

Skin barrier function assessment: Electrical impedance spectroscopy is less influenced by daily routine activities than transepidermal water loss

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Skin barrier function assessment is commonly done by measuring transepidermal water loss (TEWL). An important limitation of this method is the influence of intrinsic and extrinsic factors. Electrical impedance spectroscopy (EIS) is a lesser-established method for skin barrier function assessment. Some influential factors have been described, but no guidelines exist regarding the standardization of these measurements. Therefore, we evaluated the effect size of everyday daily routine activities on EIS and TEWL measurements as well as their correlation with age and anatomical differences. Healthy participants (n=31) were stratified into three age groups (18-29, 30-49, and ≥50 years) and EIS and TEWL measurements were performed on the left and right volar forearm and abdomen. Body cream application significantly decreased TEWL (15': $p<0.0001$; 90': $p=0.0077$) and EIS values (15': $p<0.0001$; 90': $p=0.0015$). Skin washing decreased TEWL after 15 minutes ($p<0.0001$) and EIS values for 15 ($p<0.0001$) and at least 90 minutes ($p<0.0001$). TEWL was significantly increased 5 minutes after moderate to intense exercise ($p=0.0007$). Coffee intake significantly increased TEWL on the abdomen after 60 minutes ($p=0.0146$). TEWL and EIS values did not correlate with participants' age and no anatomical differences were observed. Body cream application and skin washing should be avoided at least 90 minutes prior to measurements of TEWL and EIS. Exercise and coffee intake should also be avoided prior to TEWL measurements. EIS may be a promising tool for skin barrier function assessment as it is less affected by daily routine activities than TEWL.

LB1714

Increased epidermal penetration in aged skin modulates systemic inflammation

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Systemic inflammation, associated with impaired epidermal barrier function, increases starting in middle age. However, transepidermal water loss (TEWL), the traditional measure of "inside-out" barrier function, remains intact in middle-aged and aged skin. We hypothesized that an additional source of age-related inflammation is a defective barrier to outside agents ("outside-in"). We first compared epidermal permeability to outside agents in untreated newborn vs. aged human epidermis, using a previously validated fluorescent tracer, Ca2+ Green. Permeability, as quantified by penetration volume, was 2-3x higher in aged skin. Since occlusion does not increase inflammation in young skin but increases penetration of agents on the surface of the stratum corneum (SC), we hypothesized that using occlusion to increase penetration through a defective barrier in aged skin would induce inflammation in middle-aged (12 mos) but not young (8-12 weeks) mice. Occlusion decreased SC hydration but did not change TEWL or the immune cell profiles in both young and aged mice. Occlusion increased serum IL-1 beta and IL-6, both known to be derived from epidermis, to a much greater extent in middle-aged mice and significantly enhanced Ca2+ Green permeability in middle-aged but not young mice. Occlusion did not increase serum TNF-alpha, consistent with the minimal changes seen in immune parameters. Finally, we demonstrated that larger but more widely spaced, experimentally induced barrier defects that mimic those seen with decreased hydration lead to increased outside-in penetration without increasing TEWL. These findings suggest that an "outside-in" mechanism, linked to hydration defects, might induce systemic inflammation derived from aging epidermis.

LB1715

Beyond established protection: Adaptive epidermal barrier functions regulate systemic energy metabolism and body composition

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The skin provides a life-sustaining structural and immunological barrier for the organism. A defective epidermal barrier is a common feature of various human skin disorders, including ichthyosis, atopic dermatitis, and psoriasis. Notably, these inflammatory skin diseases are prominent examples of skin diseases associated with serious comorbidities such as short stature, obesity, arthritis. However, the functional relationship between epidermal barrier defect and systemic comorbidity is little understood. Recently, we generated a mouse model with epidermal-specific mTORC2 inactivation (Ric^{EKO} mouse) that resembles human ichthyosis in many aspects. In new studies, we now demonstrated that epidermal barrier defects in Ric^{EKO} mice are associated with prominent systemic alterations. To adapt to the cold environment, the Ric^{EKO} neonate remolds brown adipose tissue morphogenesis. Further, in adulthood, mice exhibit increased energy expenditure, need higher glycolytic fuel utilization, and show increased lipolysis to compensate for the energy loss. These altered metabolic processes in turn lead to growth retardation, lean phenotype, resistance to obesity, and altered lipidome profiles in fat depots. Together, our findings highlight an important link between epidermal barrier function and systemic metabolism at multiple levels which might contribute to comorbidity pathology. We propose the Ric^{EKO}mouse as an interesting preclinical disease model to untangle a functional communication between skin barrier defect and whole-body metabolism.

LB1716

Dipotassium glycyrrhizinate inhibits hyaluronidase activity and preserves hyaluronic acid level under UVB stress, contributing to skin moisture protection and aging fighting benefits

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Hyaluronic acid (HA) is a key molecule involved in binding and retaining water in skin. Previous research has shown that HA downregulation during photoaging may be due to changes in the expression and function of its degradation enzymes, the hyaluronidases (HYALs). In our research, we utilized the 3D-cultured human epidermal model and found that UVB irradiation could lead to HA degradation. Dipotassium glycyrrhizinate (DG), is widely known for its anti-inflammatory properties in skin care, however, its effect on skin hydration, particularly on HA, has not been previously studied. Our results showed that 0.1% DG helps preserve HA level after UVB exposure with a protection ratio up to 91.8%. Furthermore, we showed that DG could inhibit hyaluronidase activity in a dose dependent manner. Rice ferment filtrate (Rice Power™ No.11α)[1] is known to improve the skin's moisturizing ability and skin barrier function to the level of naturally healthy skin due to increasing hyaluronic acid and other efficacies. Considering the possible synergy effect of DG and rice ferment filtrate on HA, we designed topical treatments containing these two to evaluate the treatments' benefits in hydration both *in vitro* and *in vivo*. We showed the treatment could increase HA levels by 40% in a 3D skin model. And in clinical, we observed significant hydration improvement up to 72hrs after single treatment application compared to untreated skin. In a 4-hour dry and cold environmental challenge study, the skin hydration was kept at a significant higher level when compared to untreated skin and baseline. In a randomized controlled trial comparing cream and lotion regimes with lotion alone, both treatments improved skin hydration, elasticity, smoothness, texture, and reduced wrinkles and lines over 8 weeks. The regime showed significant anti-aging improvement compared to the lotion alone treatment as early as 1 week. These results demonstrate that these cosmetic treatments not only relieve skin dryness, but also provide benefits towards anti-aging.