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Nrf2 activation enhances cutaneous wound healing by expansion of hair follicle stem cell populations

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The transcription factor Nrf2 is a key regulator of the cellular stress response through the regulation of antioxidant enzymes, cytoprotective proteins and various transporters. Pharmacological activation of Nrf2 is therefore a promising strategy for both skin protection and cancer prevention. Here we show that genetic activation of Nrf2 in keratinocytes enhances the closure of full excisional back skin wounds through an increase in the length and area of the wound epithelium. Surprisingly, Nrf2 activation had no influence on proliferation and the closure of full excisional back skin wounds through an increase in the length and area of the wound epithelium. Importantly, Nrf2 activation specifically expanded junctional zone and upper isthmus stem cells among the hair follicle stem cell populations, which were shown to migrate into the wound and form the wound epithelium. A longer epithelium following tape stripping of the dermis confirms a potent inhibitor of endogenous follicular stem cells. Concerns from enhanced re-epithelialization after genetic Nrf2 activation. Nrf2 mediated the proliferation of hair follicle stem cells by increased EGFR signaling as a consequence of an up-regulation of the EGFR family member Epig, which we recently identified as a direct target of Nrf2. Thus, Nrf2 activation mediates the expansion of junctional zone and upper isthmus hair follicle stem cells by enhancing EGFR signaling. This provides a larger pool of available keratinocytes, which can migrate into the wound and form a longer wound epithelium after wounding. Pharmacological Nrf2 activation, thus appears to be a promising approach for the enhancement of wound healing through the expansion of hair follicle stem cell pools.

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Combined loss of integrins α1β1 and α2β1 results in reduced angiogenesis in mice

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Loss of integrin α1β1 in mice results in reduced tumor size and tumor angiogenesis, whereas expression of the opposite condition in wounds and tumors suggests that integrin α1β1 and α2β1 have opposing effects on angiogenesis. However, the syngeneic outcome of the opposing effects of these two integrins in angiogenesis remains elusive. We analyzed wound and tumor vascularization in mice with constitutive ablation of α1β1 and α2β1, thereby removing the α1β1 and α2β1 receptors (dKO). Compared to controls, dKO mice displayed reduced wound angiogenesis at day 3 and 7 post wounding. These results were confirmed by reduced angiogenesis of sponges implanted into the thoracic cavity of dKO mice compared to controls. This reduction was a result of increased levels and activity of matrix metalloproteinase-9 (MMP-9), thatcleaves circulating plasminogen to release increased amounts of plasmin, which increased fibrinolysis via plasminogen-dependent mechanisms.

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Complex skin models and impedance spectroscopy as new tools for dermatological research

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The aim of this study was to improve current tests based on in vitro skin models both regarding a higher in vivo correlation and analytical methods. Despite advances in the development of in-vitro-tissue-models such as reconstructed human epidermis (RHE), the questions in dermatological research, which can be addressed with the models is limited. This is due to a lack of key cellular components and a restricted live time of the models. In addition, the analysis of the models is still dependent on invasive methods such as histological processing or MTT staining. To overcome these pitfalls, we achieved advanced culture systems and biomaterials which allow full term culture of complex tissue-equivalents. Using these technologies, we have developed the first full thickness skin-model with a perfused vascular network. Furthermore, we demonstrated an alternative novel non-destructive methods, where we established a non-destructive to analyze the integrity of the epidermal barrier based on impedance spectroscopy. RHE typically exhibits characteristic impedance spectra in a frequency ranging between 1 Hz and 100 kHz, which is comparable to the spectra of freshly isolated human epidermal biopsies. From these spectra, we extracted electrical parameters of the RHE such as the capacitance and the ohmic resistance. These parameters change significantly during epithelial differentiation and were used to quantify the effects of mechanical and chemical disruption of the epidermal integrity. Most relevant, impedance spectroscopy shows a sufficient sensitivity to detect a transient decreased ohmic resistance caused by 2-propanol, which is classified as a non-irritant by MTT assays. This result indicates that impedance spectroscopy can be employed as an additional method to assess mild irritative effects. In our work we could create new technologies for the generation and analysis of tissue-models which is a vital requirement to value of in-vitro-test-models for dermatological research.

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Epigenetically regulated caveolin-1 is responsible for hyper-responsiveness to mechanical stimuli and the activation of fibroblastogenic-related RUNX2 in keloid fibroblasts

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Keloids are pathological scars characterized by excessive extracellular matrix production and prone to form in body sites with increased skin tension. We recently found that keloid fibroblasts autonomously exhibit non-spatially distributed filament elasticity and enhanced non-directional migration. Caveolin-1 (Cav-1) is the principal coat protein of caveolea functioning as scaffolds for mechanosensing. In this study, we assessed the causal relationship between Cav-1 expression and fibrosarcoma receptor-mediated responses to mechanical stimuli in keloid fibroblasts. Using atomic force microscopy, we found that keloid fibroblasts were softer than control fibroblasts, and showed a loss of stiffness sensing characterized by a failure to adjust their stiffness in response to that of the matrix. The loss of Cav-1 also contributed to the hyperactivation of fibroblastogenic-related RUNX2, a transcription factor germane to intercellular/extracellular organization, and increased migratory ability in keloid fibroblasts. The augmented HDAC-2 in keloid fibroblasts was responsible for the downregulation of Cav-1 and the hypo-responsiveness to mechanical stimulation. These results may provide a novel role for Cav-1 down-regulation in linking aberrant responsiveness to mechanical stimulation with the progression of keloid disease, findings which may lead to new developments in disease diagnostics, prophylactics, and therapeutics.

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Systemic HMGB1 administration ameliorates bleomycin-induced skin fibrosis by promoting accumulation of bone marrow-derived mesenchymal stem cells to the lesion

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Bone marrow-derived cultured mesenchymal stem cells (MSCs) have been documented to alleviate various diseases by their paracrine and immunomodulatory effects. Our recent studies revealed that systemic HMGB1 administration could enhance the accumulation and migration of MSCs functionally impaired by bleomycin-induced skin fibrosis. Here we aimed to develop new specific preparation methods to characterise the biochemistry of FRMs using mass spectrometry (MS). We extracted and isolated FRMs from human umbilical cord tissue (n=3). 558

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Advances in extracellular matrix proteomics through optimised sample preparation

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Fibrin-rich microfibres (FRM) exist both as independent tissue components: (i) in the eye and the skin (papillary dermis) and; (ii) in association with elastic fibres (reticular dermis). Proteins from the papillary dermis acts as an early biomarker of UV-mediated tissue remodelling. We have shown previously that FRM ultrastructure is sensitive to solar simulated radiation. However, it is not known if UV radiation also affects FRM protein composition or fibrillin-1 structure. The proteomic analysis of many extracellular matrix assemblies is hampered by their tightly folded structure as junk cleavage sites. In this study we aimed to develop new specimen preparation methods to characterise the biochemistry of FRMs using mass spectrometry (MS). We extracted and isolated FRMs from human umbilical cord tissue (n=3). We treated fresh FRMs with TCA/acetone to solubilise followed by trypsin digestion; i) solubilisation followed by eluate digestion and; ii) high temperature ThermoScientific LC/MS/MS. Samples were run on an Orbitrap EQ/C212. Compared to conventional trypsin digestion: i) eluate digestion increased the 1st amino acid coverage of fibrillin-1 (8% to 38%) and; ii) SMART Digestion enhanced the detection of FRM-associated proteins (from 3 to 9). Thus, these novel digestion methods improved the protein composition of our biopsies and are definitive for FRM research.

From these results, we observed a significant increase in the number of keratinocytes in the wound epithelium. Interestingly, Nrf2 activation specifically expanded junctional zone and upper isthmus stem cells among the hair follicle stem cell populations, which were shown to migrate into the wound and form the wound epithelium. A longer epithelium following tape stripping of the dermis confirms a potent inhibitor of endogenous follicular stem cells. Concerns from enhanced re-epithelialization after genetic Nrf2 activation. Nrf2 mediated the proliferation of hair follicle stem cells by increased EGFR signaling as a consequence of an up-regulation of the EGFR family member Epig, which we recently identified as a direct target of Nrf2. Thus, Nrf2 activation mediates the expansion of junctional zone and upper isthmus hair follicle stem cells by enhancing EGFR signaling. This provides a larger pool of available keratinocytes, which can migrate into the wound and form a longer wound epithelium after wounding. Pharmacological Nrf2 activation, thus appears to be a promising approach for the enhancement of wound healing through the expansion of hair follicle stem cell pools.