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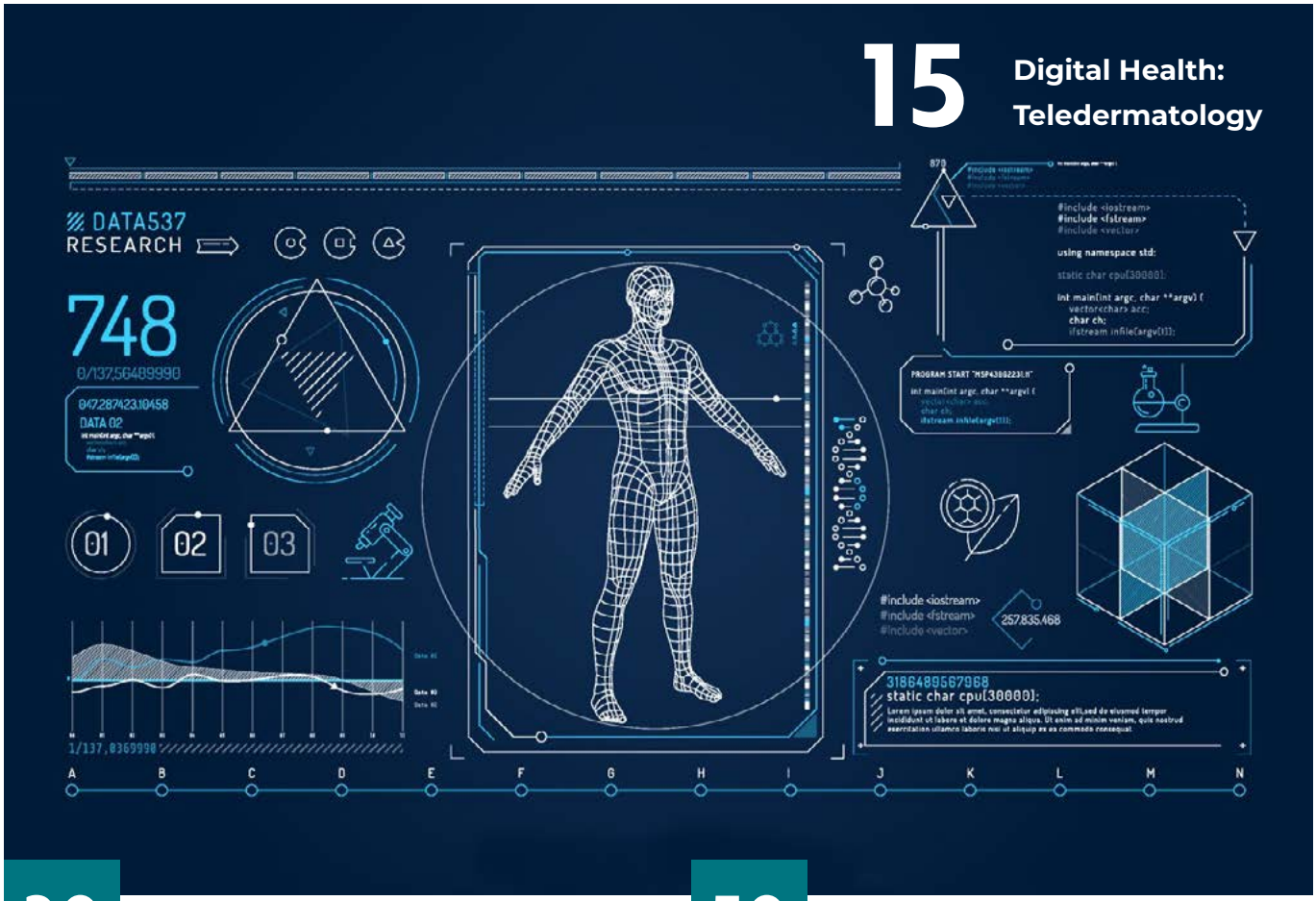
DERMATOLOGICA HELVETICA



Société Suisse de Dermatologie et Vénéréologie
Società Svizzera di Dermatologia e Venereologia
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Schweizerische Gesellschaft für Dermatologie und Venerologie

WHAT'S NEW

NRF3 suppresses squamous carcinogenesis, involving the unfolded protein response regulator HSPA5

This section is a contribution from the SKINTEGRITY.CH interdisciplinary research consortium. The present work was performed by Dr. Selina Gurri and colleagues, in a collaboration that included the SKINTEGRITY.CH Biobank as well as consortium PIs Profs. Reinhard Dummer, Daniel Hohl and Sabine Werner.

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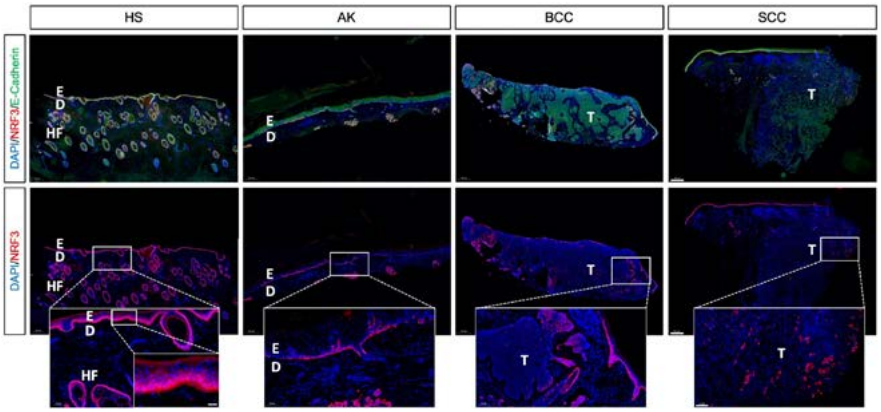

Figure 1: Downregulation of NRF3 protein in NMSC. Representative NRF3 immunofluorescence stainings of sections from normal human skin (HS), AK, BCC, or SCC using antibodies against NRF3 (red) and E-cadherin (green). Nuclei were counterstained with Hoechst (blue). Scale bar: 500 µm. The areas indicated with a rectangle are shown at higher magnification in the indent. Scale bar: 100 µm or 20 µm. D, dermis; E, epidermis; HF, hair follicle; T, tumor.

Selina Gurri. Non-melanoma skin cancer (NMSC), which mainly includes basal and squamous cell carcinomas (BCCs and SCCs), ranks as the most common cancer among humans, affecting 2-3 million people every year. By far the most common risk factor is UV irradiation, responsible for approximately 90 % of all cases. With the population's increasing sun exposure and the steady degeneration of the ozone layer, NMSC incidences continue to increase. Therefore, it is crucial to identify key players in skin carcinogenesis and to assess their potential as drug targets. In 2018, our group showed that the CNC basic leucine zipper transcription factor NRF3 promotes UVB-induced apoptosis of keratinocytes. NRF3 is the newest and least researched NRF family member, and therefore, its activation, regulation and physiological functions are still poorly characterized. Given the important function of the related cytoprotective NRF2 protein in skin cancer and the key role of UV radiation in the pathogenesis of these malignancies, the finding of NRF3's role in the UV response of keratinocytes prompted us to investigate a potential role of this protein in epithelial skin cancer.

Using immunofluorescence staining for NRF3, we found reduced NRF3 protein levels in actinic keratosis (AK) skin cancer precursor lesions compared to normal skin and an almost complete loss of this protein in invasively growing tumor cells of BCCs and SCCs *(Figure 1).* We found that the loss of NRF3 strongly promoted the malignancy of skin cancer cells in 2D and 3D cell culture models as reflected by their enhanced clonogenicity, faster migration, formation of bigger spheroids and highly invasive growth into the dermal equivalent in 3D organotypic skin cultures. *In vivo,* NRF3-deficient skin cancer cells showed increased malignant growth in xenograft and toxin-induced mouse skin cancer models. These data demonstrate a potent tumor-suppressive function of NRF3 in the skin.

Mechanistic studies, involving RNA-sequencing and proximity-dependent Biotin Identification (BioID) screening, revealed that the tumor-suppressive effect of NRF3 is most likely not mediated by its activity as a transcription factor, but likely results from the interaction of NRF3 with HSPA5. HSPA5 is the master regulator of the unfolded protein response (UPR) in the endoplasmic reticulum (ER). Interestingly, an elevated UPR activity and increased HSPA5 levels are a hallmark of many cancer cells and correlate with cancer cell survival and malignancy.

Upon loss of NRF3, HSPA5 protein levels were increased under ER stress and homeostatic conditions, while HSPA5 mRNA levels were not significantly changed, indicating that loss of NRF3 stabilizes HSPA5. Additionally, HSPA5 was also highly expressed in cutaneous BCCs and SCCs, especially in the invasively growing skin cancer cells, which showed low or no NRF3 expression. This suggests an important role of the NRF3-HSPA5 interaction in the tumor-suppressing effect of NRF3 in the skin. Indeed, pharmacological inhibition or knock-down of HSPA5 rescued the malignant features of NRF3-deficient cancer cells *in vitro* and in preclinical mouse models. These results suggest HSPA5 inhibition as a promising treatment strategy for NMSC in stratified cancer patients.

Taken together, we discovered that NRF3 protein levels are downregulated in NMSC and that NRF3 acts as a potent tumor-suppressing protein in the skin. This involves the unfolded protein response regulator HSPA5, which we identified as promising target for the treatment of NMSC *(Figure 2)*.

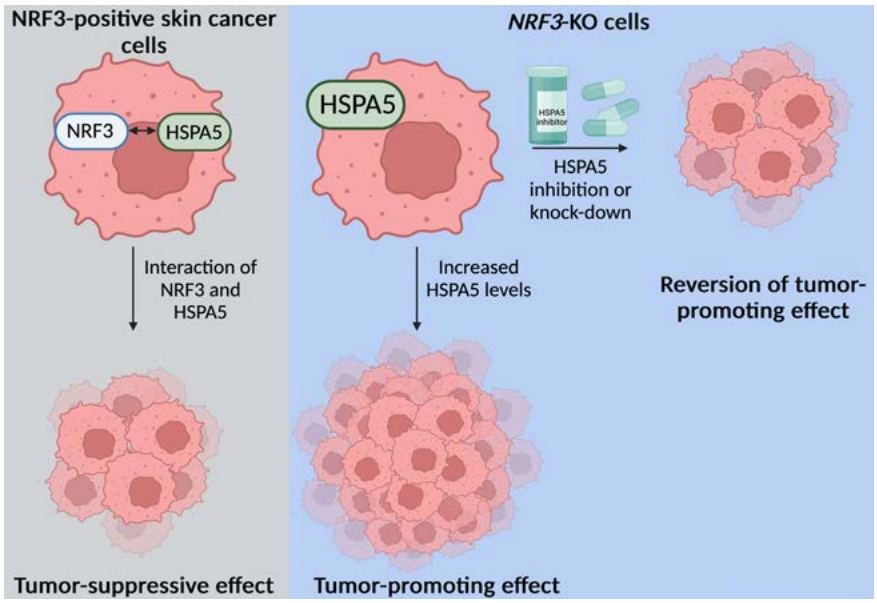

Figure 2: Model summarizing the tumor-suppressing effect of NRF3 in the skin through the involvement of HSPA5. In NRF3-positive skin cancer cells, NRF3 acts as a tumor-suppressing protein by interacting with HSPA5, thereby reducing its abundance. In NRF3-KO cells, HSPA5 levels are increased due to the missing regulation by NRF3, resulting in enhanced cancer cell malignancy and tumorigenesis. Treatment with HSPA5 inhibitors or knock-down of HSPA5 suppresses the tumor-promoting effects of NRF3 deficiency. Created with BioRender.com.

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Reference

1. *Gurri S., Siegenthaler B., Cangkrama M., Restivo G., Huber M., Saliba J., Dummer R., Blank V., Hohl D., & Werner S. (2023). NRF3 suppresses squamous carcinogenesis, involving the unfolded protein response regulator HSPA5. EMBO molecular medicine, e17761. https://doi.org/10.15252/emmm.202317761*