

# Skin Biology



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## Genodermatoses as a clue to cancer development

Epidermodysplasia verruciformis (EV) and ichthyosis with confetti (IWC), are genodermatoses serving as models for non-melanoma skin cancer (NMSC) development. Elucidating the mechanisms of these diseases will help to understand NMSC development in the general population.

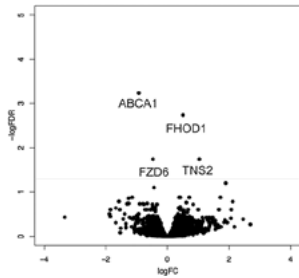
Epidermodysplasia verruciformis (EV) is an autosomal recessive disease. EV patients develop plane wart-like lesions in childhood mainly on the neck and extremities. Most patients develop NMSC, mostly on UV-exposed areas. They are susceptible to beta-human papilloma virus (HPV). These HPV are harmless to the general population because they miss *E5*, a gene which is probably necessary for HPV to overcome the immune system. In about 50% of patients, biallelic null mutations are found in *TMC6/EVER1* or *TMC8/EVER2*. Function of both proteins and the pathomechanism in EV are unknown. It is assumed that they are part of keratinocyte-intrinsic immunity, since EV patients are not susceptible to other infectious diseases.

We have expanded the phenotypical spectrum of EV by careful examination of patients. In collaboration with Casanova's group (Rockefeller University, NY; INSERM and Imagine Institute, Paris) we discovered *CIB1* (calcium and integrin binding 1) as a novel EV-associated gene. Functional studies showed the formation of a complex of both TMCs and CIB1. We generated a keratinocyte model using CRISPR-Cas9 enabling future functional studies of CIB1 independently of patient material. Ichthyosis with confetti (IWC) is an autosomal dominant hereditary skin disorder. IWC patients suffer lifelong from erythroderma, but develop pale spots within the inflamed skin area, which look like healthy skin. All patients are carrier of a heterozygous keratin (*KRT*) 1 or *KRT10* mutation which leads to a shifted reading-frame. We showed that the reading frame needs to be translated in an arginine-rich C-terminus of the keratin, leading to its nuclear accumulation instead of cytoplasmic localization (Fig. 2). It needs to be elucidated how the nuclear localization is associated with the disease-typical mitotic recombinations or gene conversions affecting the chromosome with the mutated KRT. This loss of heterozygosity leads to keratinocytes expressing the wildtype allele only. These cells present as pale spots on the skin. By clinical examination of patients and next-generation sequencing on fixed material from deceased patients, we showed that IWC patients have an increased risk of NMSC. Further functional analyses will be done to clarify how the IWC-causing pathomechanisms are related to NMSC and whether the same mechanisms are involved in NMSC in the general population.

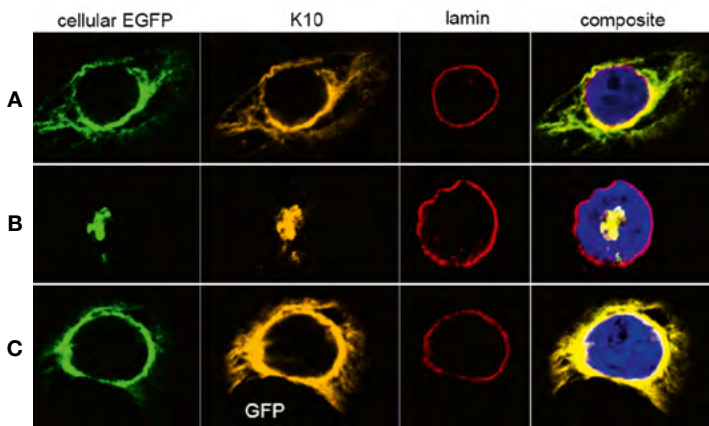
As model to study skin diseases, we developed epidermal organoids. An organoid is a collection of organ-specific cells that develops from stem cells or organ progenitors and has the capability to recapitulate the function and architecture of the target organ. They are used for drug development and toxicity studies and in personalized medicine. They also have the potential to replace organ transplantation. In contrast to 2D keratinocyte cultures, epidermal organoids recapitulate the function and structure of the epidermis. By testing culture conditions, we have established Murine and Human Epidermal Organoids (HEO). HEO could be successfully generated from human foreskin keratinocytes (Fig. 3) and skin biopsies from healthy donors or patients.

While being necessary to protect us against dangers from the environment, innate immunity in the skin, when dysregulated, leads to various inflammatory dermatoses. Due to the complexity of innate immune responses and the plethora of insults our skin has to face, the physiology of inflammatory skin diseases is often not well known. Our current research projects are aimed at investigating the molecular events driving exacerbated innate immune reactions in the skin with a spe-

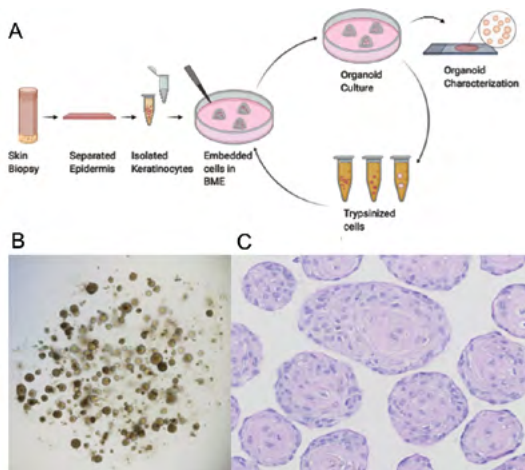
cial focus on hard to treat diseases such as rosacea, atopic dermatitis, pyoderma gangrenosum and hidradenitis suppurativa. Also, we are investigating the role of innate immunity in life threatening complications occurring after intake of certain medications, namely severe cutaneous adverse drug reactions.



**Fig. 1:** RNAseq analysis of a high number of CIB1 knockouts with isogenic background showed small changes in expression level of few genes (Figure adapted from Imahorn *et al.*, 2020).



**Fig.2:** Localization of various N-terminal GFP-labelled keratin 10 (K10) following transient transfection of a keratinocytes with plasmids encoding K10 with different C-termini. K10 was either detected by imaging of eGFP expressed from plasmid (cellular eGFP, green) or immunostaining with an anti-K10 or anti-GFP antibody (orange). The nuclear membrane was labelled with an anti-lamin antibody (red). DAPI (blue) was used for nuclear staining. **A.** K10 wildtype is localized in the cytoplasm of the keratinocyte. **B.** K10 with the IWC-typical arginine-rich C-terminus is localized in the nucleus of transfected keratinocytes. **C.** K10 with an alanine-rich C-terminus as the result of the alternative third reading frame is clearly localized in the nucleus. Immunostaining of K10 alanine was done using the anti-GFP antibody since this K10 was not detected by the anti-K10 antibody. (Figure adapted from Renz *et al.*, 2019).



**Fig.3:** Generation of human epidermal organoids recapitulating the epidermis structure.

**A.** Overview of the method. **B.** Human organoids growing in Matrigel (light microscopy). **C.** Hematoxylin & Eosin coloration of human epidermal organoids after 7 days of culture.

## Connection to Clinical Practice

**Prof. Dr. P. Itin (retired during period; laboratory head Dr. Burger)**  
**Prof. Dr. A. Navarini ( laboratory head Dr. Contassot)**

Dermatology Department, USB

### Skin inflammation in drug reactions, rosacea and other skin conditions

Until July 2020, the aim of our research was to identify mutations driving rare skin diseases and functionally investigate the proteins and their link to the phenotype observed in patients. Our research group is in transition due to the change of the research group leader. Whilst the aim of our research remains the same, namely to understand dermatologic conditions on the level of the pathophysiology, our focus is moving towards immunology. To this end, the labhead has changed from Dr. Bettina Burger to PD Dr. Emmanuel Contassot on the 1<sup>st</sup> of July 2020. We are currently building up research project involving the pathophysiology of drug reactions and the inflammatory skin disease rosacea. To this end, we closely work with the Dermatology Department to: Investigate the relevance of biological and genetic observations using biological material from patients and characterise the molecular mechanisms that mediate pathogenic events and their link with lesion development in patients.

### Selected Publications

- Imahorn E, Aushev M, Herms S, Hoffmann P, Cichon S, Reichelt J, Itin PH and Burger B (2020). Gene expression is stable in a complete CIB1 knockout keratinocyte model. *Sci Rep* 10, 14952.
- Burger B, Ghosh A, Ng CKY, Piscuoglio S, Spoerri I, Itin PH, Greer K and Elbaum D (2020). Discovery of heterozygous KRT10 alterations in MAUIE cases underlines the importance of regular skin cancer screening in ichthyosis with confetti. *The British journal of dermatology*.
- Renz P, Imahorn E, Spoerri I, Aushev M, March OP, Wariwoda H, Von Arb S, Volz A, Itin PH, Reichelt J *et al.* (2019). Arginine- but not alanine-rich carboxy-termini trigger nuclear translocation of mutant keratin 10 in ichthyosis with confetti. *J Cell Mol Med* 23, 8442–8452.
- de Jong SJ, Crequer A, Matos I, Hum D, Gunasekharan V, Lorenzo L, Jabot-Hanin F, Imahorn E, Arias AA, Vahidnezhad H *et al.* (2018a). The human CIB1-EVER1-EVER2 complex governs keratinocyte-intrinsic immunity to beta-papillomaviruses. *J Exp Med* 215, 2289–2310.
- Imahorn E, Yuksel Z, Spoerri I, Gurel G, Imhof C, Saracoglu ZN, Koku Aksu AE, Rady PL, Tying SK, Kempf W *et al.* (2017). Novel TMC8 splice site mutation in epidermodysplasia verruciformis and review of HPV infections in patients with the disease. *J Eur Acad Dermatol Venereol* 31, 1722–1726.