Flower: a tiny protein with a big job in skin biology

Authors: Eline Geervliet, Suzie Grigorjan, Susan Roelofs

Our skin may seem simple on the surface, but beneath it lies an incredibly coordinated system that protects us from dehydration, harmful substances, and environmental stress. This system depends on precise communication, vesicle transport, and structural remodeling.

In recent years, researchers have uncovered new players involved in this mechanism of communication, transport and remodeling, and one of the most intriguing is a small membrane protein called Flower (FWE). In this 3-part blog series, we take a closer look at the skin barrier and unravel the functions of the FWE protein in depth. In episode 1, we explore how the epidermis, the outermost protective layer of the skin, is organized and how keratinocytes mature as they travel through its layers. Understanding this foundation prepares us for episode 2, where we dive into FWE's role in vesicle transport and calcium regulation. Building on this, episode 3 takes us into how disrupted FWE function may contribute to skin disorders.

Previously

In the first episode of this series we have learned more about the role of each layer of the epidermis. The role of tight junctions, lamellar bodies and corneocytes in the epidermal barrier and the importance of calcium as master regulator also became clear. In this blog we will discuss the Flower protein: a tiny protein with a big job in skin biology.

What is flower

The Flower (FWE) gene encodes four small transmembrane proteins known for their roles in vesicle trafficking and calcium-dependent membrane dynamics. The FWE gene has been investigated in several cell types in mice. In nerve cells, the FWE protein is found on tiny sacs called synaptic vesicles. It helps take these sacs back into the cell after they release their contents and makes sure the process of releasing and retrieving vesicles works smoothly together [1], [2]. In specific immune cells, the cytotoxic T lymphocytes, FWE helps control how lytic granules, specific type of vesicles, are taken back into the cell after they release their contents [2], [3]. Both functions are found to be calcium dependent. Rudd et al. explored how FWE controls the movement and release of lamellar bodies (LBs) and how this process helps build the epidermal barrier. They also investigated the importance of calcium in this process [2].

FWE localization

To investigate the role of FWE, localization of this protein was important. Confocal microscopy revealed that the FWE protein is present in the upper stratum spinosum (SS) and throughout the whole stratum granulosum (SG). As discussed in the previous episode, these two layers play an important role in the epidermal barrier. Co-staining with desmosomal cadherin desmoglein-1 (DSG1), a crucial cell adhesion molecule in cells, showed that FWE is accumulated just underneath the plasma membrane in the SS and deeper SG, while in the upper SG, FWE fused with the membrane (**Figure 1**). This indicates that FWE marks a specific subset of vesicles that move toward the apical surface of keratinocytes in the SG. This is precisely the region where the skin assembles its LBs and lipid-rich extracellular matrix (ECM), essential components of the barrier [2].

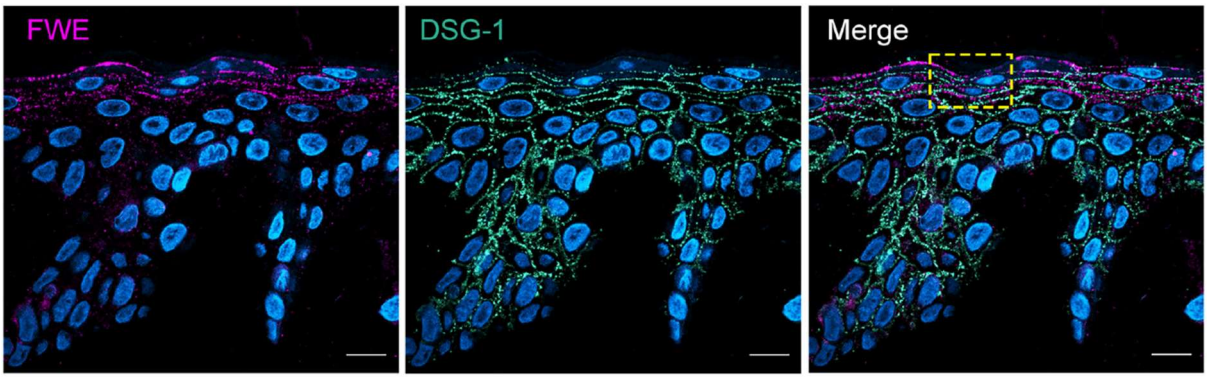

Figure 1. *Representative confocal microscopy of double immunofluorescence for desmoglein-1 (DSG-1) and Flower (FWE) in normal human epidermis (n = 3) with Hoechst-labeled nuclei (blue). Copied from [2].*

The location of FWE expression in vivo (in humans) indicates that this protein is linked to the differentiation of keratinocytes. Therefore, it was investigated if this pattern is also found in vitro (in the lab). When keratinocytes were induced to differentiate using high-calcium conditions, FWE levels rose progressively alongside established differentiation markers such as K10, loricrin, and filaggrin (**Figure 2**). This consistent upregulation further emphasizes the possible link between FWE and epidermal differentiation [2].

What happens when FWE is missing

To further evaluate the role of FWE in epidermal differentiation, barrier function was compared between FWE-expressing and FWE-deficient (cells that missed the FWE gene) models. They used a special 3D ‘air–liquid interface’ (ALI) system, which allows skin cells to grow in layers, just like real skin. In this setup, the bottom of the tissue sits in liquid, while the top is exposed to air, helping the cells form a proper skin barrier. Rudd et al. used electrical impedance

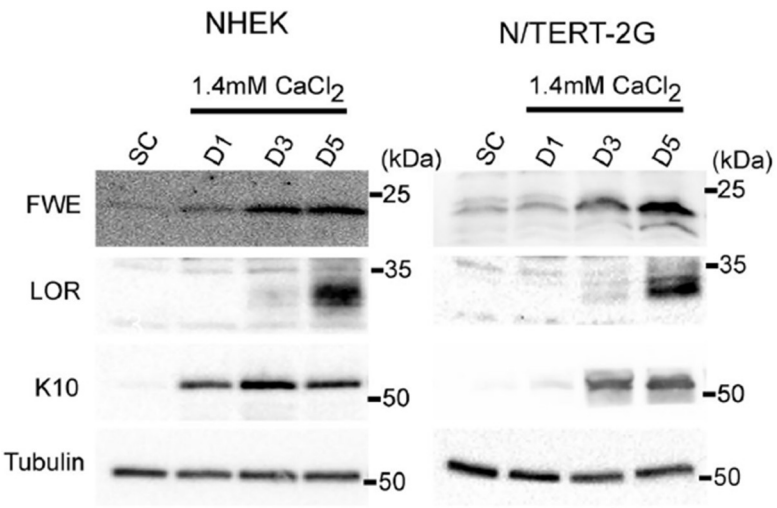

Figure 2. Representative (n = 3) immunoblots for FWE and differentiation markers in whole cell lysates collected from monolayer NHEK or N/TERT-2Gkeratinocytes in a subconfluent undifferentiated state or after differentiation in 1.4mMCaCl2 containing media for 1, 3, or 5 days post confluence. Copied from [2]

spectroscopy (EIS) to assess the barrier function. With EIS, an electrical signal is send through the skin and the signal tells how difficult it was to go through the layer: the more difficult, the better the barrier function. EIS is a non-invasive (it does not harm the cells), accurate and fast technique to evaluate (skin) barrier function [4].

As a 3D skin model matures under ALI conditions, its barrier function steadily improves, and this progression can be tracked with the area under the curve (AUC) of the EIS measurement results. In healthy models, the AUC of EIS^{diff} rose from day 1 through day 10, mirroring the natural process in which keratinocytes differentiate, assemble tight junctions in the SG, and produce the lipid matrix of the SC. Around day 10, the barrier reached its peak strength and the impedance curve stabilized or slightly declined as cornification started [4]. Interestingly, FWE expression followed a nearly identical trajectory; it increased during days 1–10, peaked in the granular layers while the skin barrier was being built, and then rapidly diminished once cornification develops (**Figure 3a**) [2].

The importance of FWE becomes especially clear when it is removed. FWE-deficient (FWE-knockout, KO) models displayed a dramatically reduced EIS^{diff} signal (**Figure 3b-c**), indicating a severely compromised barrier even though cell growth appeared normal and tissue structure showed only minor alterations (**Figure 3d**). These results suggest that FWE is not a maintenance protein but rather a key contributor during the active construction phase of the skin barrier [2].

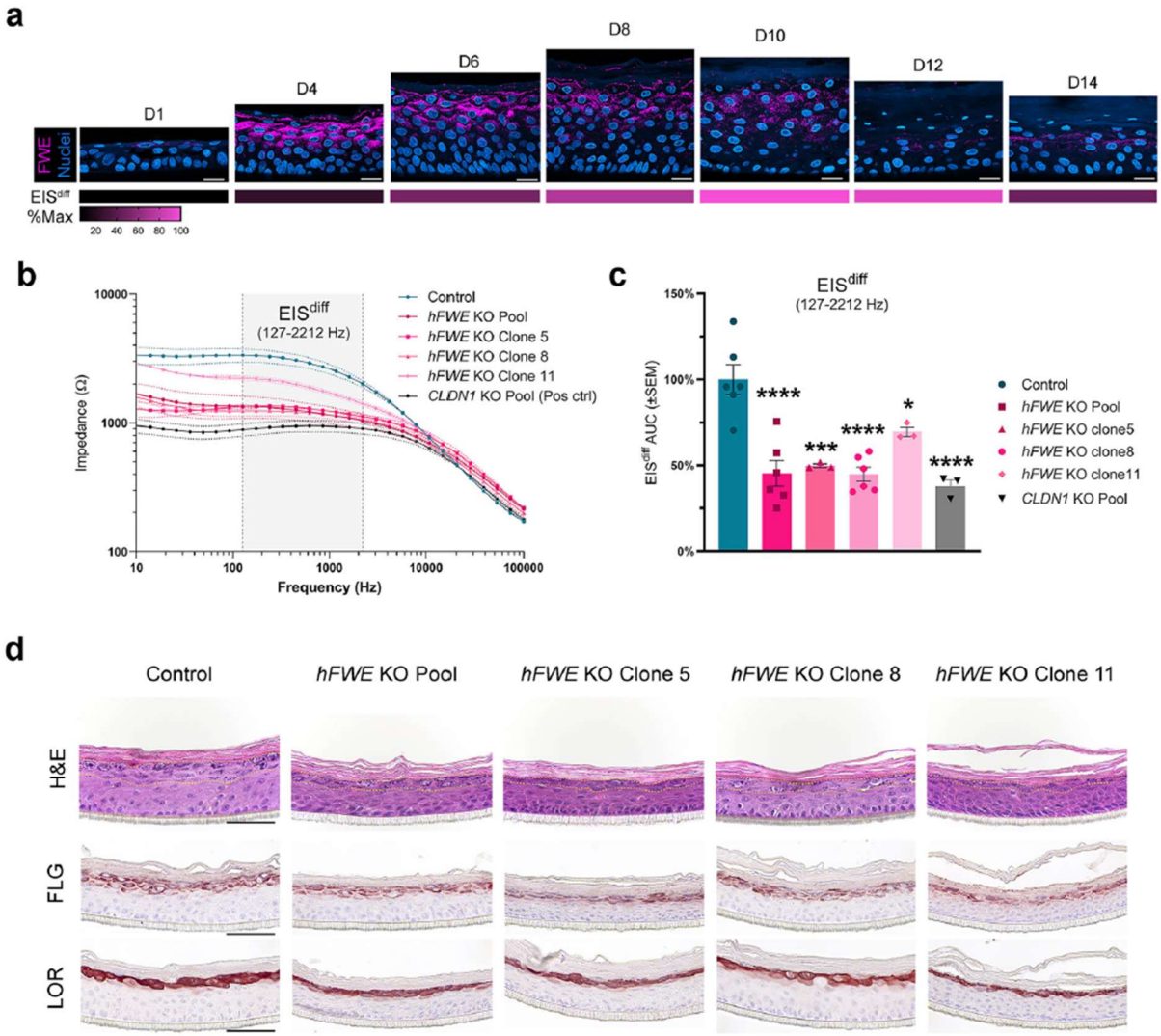

Figure 3. *a Representative FWE immunofluorescence on N/TERT-2G derived epidermal organoids grown at ALI for the indicated number of days (n =2 organoids per timepoint, scale bars, 20 µm). Heatmap beneath images reports average percentage of maximal EIS^{diff} AUC for each timepoint. b Full impedance spectra from control and hFWE KO N/TERT-2G after 10 d at ALI, as well as AUC calculations (c) for impedance values in EIS^{diff} range are shown normalized to control (* = p < 0.05, *** = p< 0.001, ****=p < 0.0001, one-way ANOVA with Bonferroni's multiple comparison test). CLDN1 KO N/TERT-2G previously developed in [4] were included as a positive control for barrier deficit. (n=6 organoids for Control, hFWE KO pool and hFWE KO clone8 over two independent experiments; n = 3 organoids for hFWE KO clone5 and clone11). d Representative (n = 3) H&E and differentiation marker (LOR, FLG) immunohistochemistry on epidermal organoids. Dashed lines on H&E indicate the border of keratohyalin granule-containing cells in the SG (scale bars, 50 µm). Copied from [2].*

FWE and lamellar bodies

The apical localization of FWE suggests a potential link to LBs, which are apically secreted in the SG. Therefore, Rudd et al. performed a co-staining of FWE with 3 distinct LB cargo molecules: corneodesmosin (CDSN), antileukoprotease (SKALP), and kallikrein-5 (KLK5), and 2 general vesicle markers: early endosome marker-1 (EEA1), and DSG1. This revealed that FWE highly co-localizes with the LB cargo molecules, while there is minimal co-localization with general vesicle markers, indicating FWE is specifically associated with LBs and not just with any vesicle [2].

In the healthy 3D skin model, the LB cargo molecule CDSN started to accumulate at day 4 and peaked in the SC around day 10, just as FWE expression declined in viable layers. In the FWE KO models, immunohistochemistry for LB markers (CDSN, SKALP) showed a reduced signal, loss of apical polarization in SG, and absence in the SC, indicating failed LB trafficking. Without FWE, LB trafficking and late-stage differentiation events appeared disrupted, preventing the epidermis from achieving full barrier integrity [2].

Rudd et al. performed a broad analysis of gene activity in the tissue, called transcriptomic profiling using bulk RNA-seq, together with a method that checks which biological programs are turned up or down, called gene set enrichment analysis (GSEA). This revealed that key drivers of the latest stages of epidermal differentiation were downregulated in FWE KO skin models. Considering FWE's established role in other organisms, where it regulates Ca^{2+}-dependent endocytosis and exocytosis of vesicles, Rudd et al. propose that in the epidermis, FWE exerts calcium-dependent control over the biogenesis and trafficking of LBs [2].

Take-home messages

- In several cell types, Flower (FWE) is involved in vesicle trafficking
- In keratinocytes, FWE is localized where lamellar bodies (LBs) are made
- FWE and keratinocyte differentiation are linked to each other
- Electrical impedance spectroscopy (EIS) is a non-invasive, accurate and fast technique to evaluate (skin) barrier function
- FWE a key contributor during the active construction phase of the barrier
- FWE is essential for guiding LBs to the skin surface. Without it, LBs can't deliver their cargo, and the epidermal barrier cannot fully form
- FWE might exerts calcium-dependent control over the biogenesis and trafficking of LB

Coming up next

In this episode we have learned that FWE is essential for epidermal differentiation and LB trafficking. In the next episode we will further explore the link between calcium and Flower.

Acknowledgement

This blog is based on the article from Justin C Rudd et al. None of this work nor data is produced by Locsense B.V. all right reserved to Justin C Rudd et al. "Flower dependent trafficking of lamellar bodies facilitates maturation of the epidermal barrier" Nature Communications , vol. 16, no. 1, Dec. 2025, doi: 10.1038/s41467-025-62105-1.

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