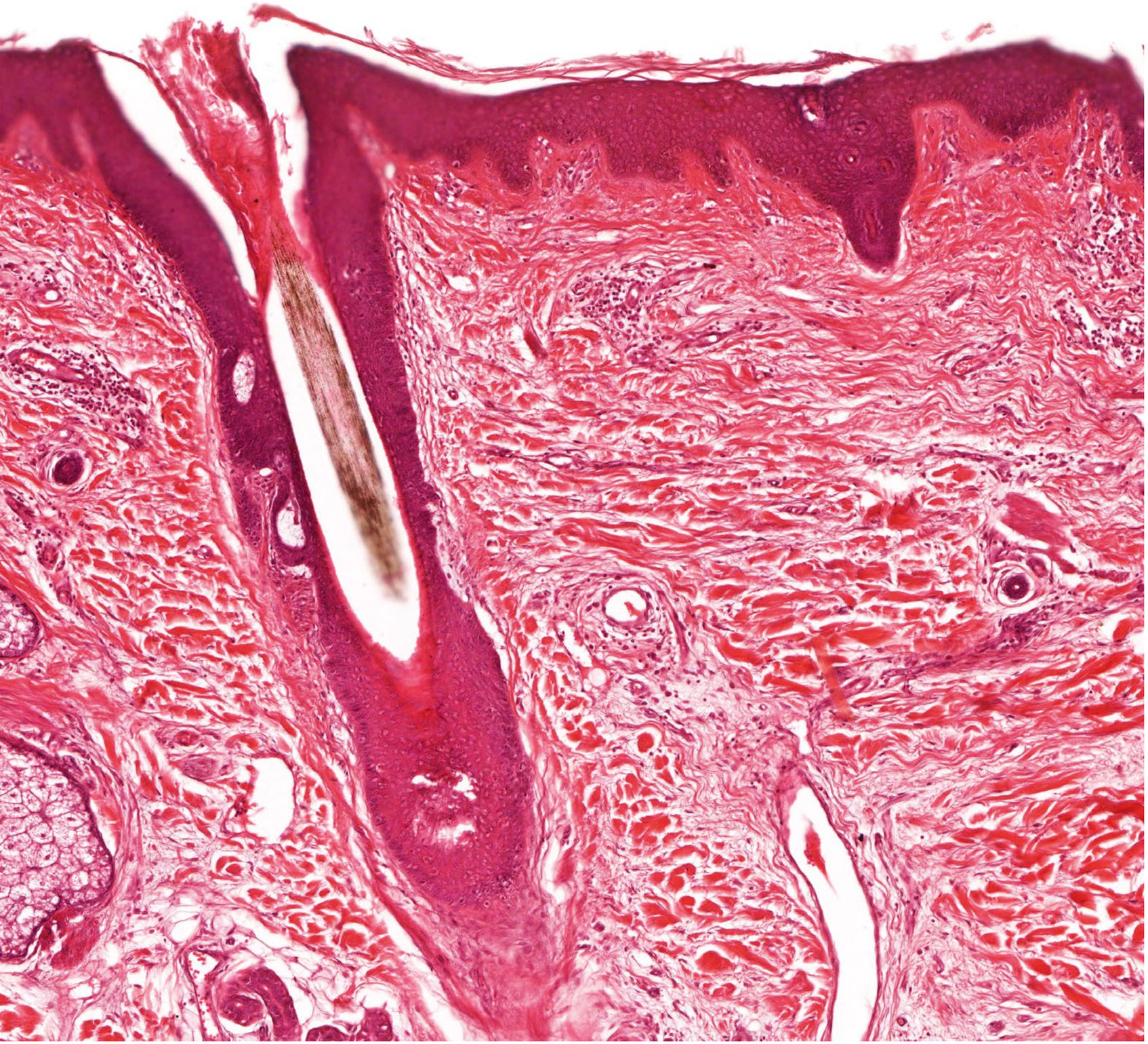


Novel mouse models reveal molecular details of skin cell death

Dr Panayotova-Dimitrova's recent work has identified a key regulator of skin homeostasis and its exact mechanisms. To elucidate this, her team employed a novel approach, using mouse models. These models enabled the researchers to dissect the role that a protein called cFLIP plays in regulating cell death in the skin, providing fresh insights into life-threatening skin diseases.

At the University of Aachen, Germany, Dr Diana Panayotova-Dimitrova leads a research group investigating the regulation of cell death pathways in the skin. Her recent work has focused on generating transgenic mouse models capable of analysing cellular FLICE-like inhibitory protein (cFLIP) in the skin, due to the major role it plays in cell death signalling.

A strong expression of the cFLIP protein in the basal layer of the human skin had been previously established by the group, indicating that cFLIP may play an important role in the development and maintenance of this tissue. Combining these factors also led the researchers to hypothesise that loss of cFLIP in skin cells may be a factor in the onset of deadly diseases related to skin cell death.

MOLECULAR PLAYERS IN CELL DEATH PATHWAYS

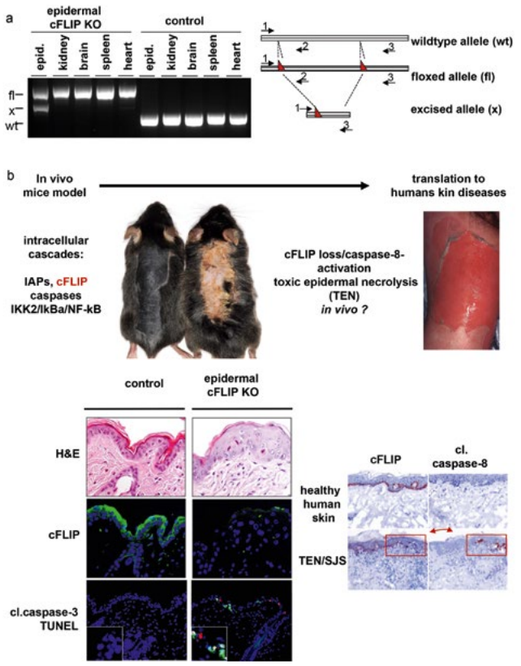
cFLIP is a regulator of apoptosis – the process of programmed cell death in response to a stress trigger or signals from other cells. Another mechanism for cell death is necroptosis, which is a form of inflammatory necrotic cell death that the cell undergoes in a regulated manner. This is in contrast to necrosis, which is uncontrolled by the cell and occurs due to cellular damage or infection.

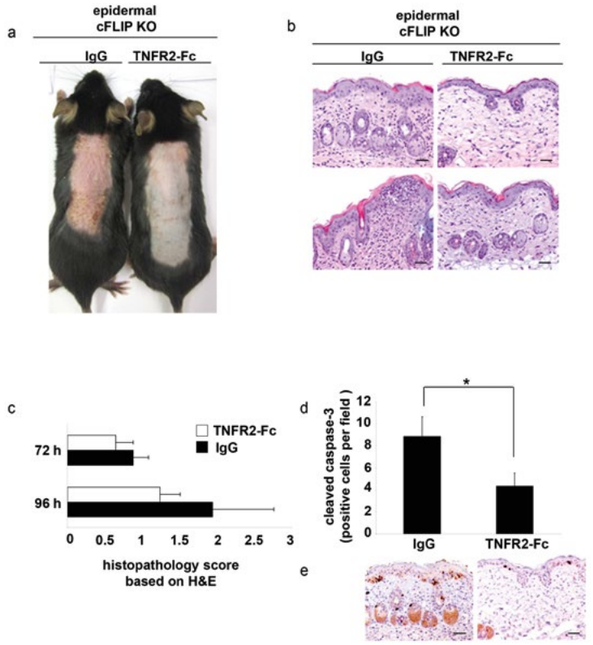
In addition to regulating the apoptotic pathway, cFLIP has also been shown to induce cell necroptosis and activate pathways associated with inflammation. Caspases are enzymes that are key players in the activation of the cell death pathway. cFLIP is a regulator of one of these essential controllers of cell death – Caspase-8 – in skin cells known as keratinocytes. These cells account for the majority of cells in the outer layer of skin called the epidermis, with around 90% of this layer made up of keratinocytes. cFLIP and Caspase-8, along with an adaptor protein (FADD), constitute a death-inducing signalling complex which forms upon activation of the death receptor.

CELL SPECIFIC GENETIC MANIPULATION

Conventional analysis of gene function is

Prior to Dr Panayotova-Dimitrova's work, virtually nothing was known about the function of cFLIP in the skin *in vivo*





Using novel mouse models, Dr Panayotova-Dimitrova's work has revealed the mechanistic importance of cFLIP in maintaining the health of epidermal skin cells and regulating skin inflammation

based on using transgenic animals where the gene of interest is either deleted or expressed in a modified form across all cells. In recent years, research employing transgenic animals has become more refined with the development of a technique for inducing these changes in a cell-specific manner. Gene function analysis can now be carried out at the level of individual cell types, as opposed to being limited to the organism as a whole. This is facilitated by Cre/loxP system-based technology, which Dr Panayotova-Dimitrova and her team have used to create their specialised transgenic mice. Cre-Lox recombination allows for DNA to be modified in target cell types, and for this modification to be consequently triggered by a specific inducer, applied externally. It is this process that Dr Panayotova-Dimitrova has used to investigate the function of cFLIP, specifically in keratinocyte skin cells.

Prior to Dr Panayotova-Dimitrova's recent work, virtually nothing was known about the function of cFLIP in the skin in vivo. This was because both conventional mouse models and those lacking cFLIP in the skin from birth have a lethal embryonic phenotype, meaning that further analysis was not possible. Fortunately, by using novel mice models, in which the cFLIP gene could be deleted after the mice had reached adulthood, the researchers were able to circumvent this obstacle.

cFLIP'S ROLE IN SKIN CELL DEATH

Dr Panayotova-Dimitrova and her team's study of transgenic mice which lacked expression of the gene from birth, resulted in embryonic lethality – indicating the crucial role cFLIP plays in tissue development. Where the cFLIP protein was removed in the skin of adults, the skin became severely inflamed, exhibiting blisters, pustules and skin loss. The researchers managed to ascertain that this was associated with caspase activation and apoptotic, not necroptotic, cell death. They also found that the apoptosis of epidermal cells that occurred in the absence of cFLIP, was dependent on autocrine tumour necrosis factor, the production of which is triggered by the loss of cFLIP.

This discovery brings with it important insights into human disease. They have found that the same loss of cFLIP is associated with severe drug reactions linked to epidermal apoptosis, such as toxic epidermal necrolysis (TEN). TEN is a severe and often fatal drug hypersensitivity reaction of the skin where excessive cell death occurs. The disease is rare, which has made understanding it more difficult. Although discoveries over the past decade have hinted at details of its pathology, the mechanism behind the reaction within the skin has eluded scientists. To date, there is no efficient treatment for TEN, and the mortality rate remains high at around 30%.

DEVELOPING LIFE-SAVING THERAPEUTICS

The phenotypes of cFLIP-deficient mouse skin closely resembled TEN skin in humans, and cFLIP loss had been already documented in patient skin cell samples. Using these novel mouse models, Dr Panayotova-Dimitrova's work has revealed the mechanistic importance of cFLIP in maintaining the health of epidermal skin cells and regulating skin inflammation. She and her team believe that cFLIP may be an effective target for therapeutic intervention to prevent excessive apoptosis – a characteristic of conditions such as TEN. Their novel insights pave the way for further research into understanding the impact of cFLIP loss, bringing with it the possibility of developing life-saving targeted therapeutics for numerous skin diseases.

Q&A

Could you tell us a bit about your prior research that led you to working on cFLIP?
I started my research on cell death signalling when I joined the group of Prof Martin Leverkus in 2007. Martin was a great mentor and outstanding scientist. He was especially interested in the regulation of cell death resistance and I was immediately fascinated by his enthusiasm working on the analysis and manipulation of cell death. The main interest of Martin's work was the regulation of death-receptor-induced apoptosis in general and with a specific focus on the role of the regulatory proteins cFLIP and cIAPs in this process. My first project was related to the characterisation of cFLIP's role in dendritic cells' maturation. When I started to work on the development of in vivo models with epidermal cFLIP deficiency, it soon became clear that I would concentrate all my efforts on this research.

What were the biggest challenges you faced in successfully developing your novel transgenic mice?
Developing and optimising the best tools for appropriate and reliable characterisation of our model was sometimes difficult.

What types of therapeutic interventions do you think could be developed to treat skin diseases such as TEN?
As we have shown in our study, cFLIP deletion leads to inflammatory and TNF-dependent keratinocytes apoptosis. Furthermore, cFLIP deletion in patients with TEN is a possible prerequisite for the fulminant cell death characteristic for this disease. These facts suggest that components, which interfere functionally with TNF signalling, such as TNF antagonists, might provide potential treatment for patients with toxic epidermal necrolysis. It was actually shown in a clinical study done by Paradisi et al in 2014 – a small group of TEN patients was successfully treated with a TNF antagonist.

What to you has been the most enjoyable aspect of being involved in this research?
Developing and analysing a new *in vivo* model can have some disadvantages. For example, *in vivo* experiments are much more time consuming compared with those done *in vitro*. However, waiting for confirmation of your hypothesis, building on *in vitro* data, and translating it to living organisms increases the excitement and the fun of working on such a project.

Where do you plan to take your research following on from this project?
We are already developing new mouse models which should help us to investigate the impact of the Ripoptosome and its downstream signals, on spontaneous and induced cell death in the skin in the context of cFLIP and its isoforms. We are also studying the involvement of additional signalling cascades, which may be involved in the development of the dramatic skin phenotype upon cFLIP deletion.

Detail

RESEARCH OBJECTIVES

Dr Panayotova-Dimitrova's research focuses on the regulation of cell death pathways in the skin. Her recent work has specifically looked into the role of the Caspase-8 regulator, cFLIP, in keratinocytes within novel mouse models.

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COLLABORATORS

- Tom Luedde, MD (University Hospital Aachen)
- Metodi Stankov, PhD (Hannover Medical School)
- Markus Rehm, PhD (University Stuttgart)

BIO

Dr Panayotova-Dimitrova received an MS in Molecular Biology before undertaking a PhD in Virology. During 2007, she joined the laboratory of Prof Martin Leverkus where she began studying the regulation of cell death. Last year, following Prof Leverkus' unexpected death, Dr Panayotova-Dimitrova took over and now leads the lab there.

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