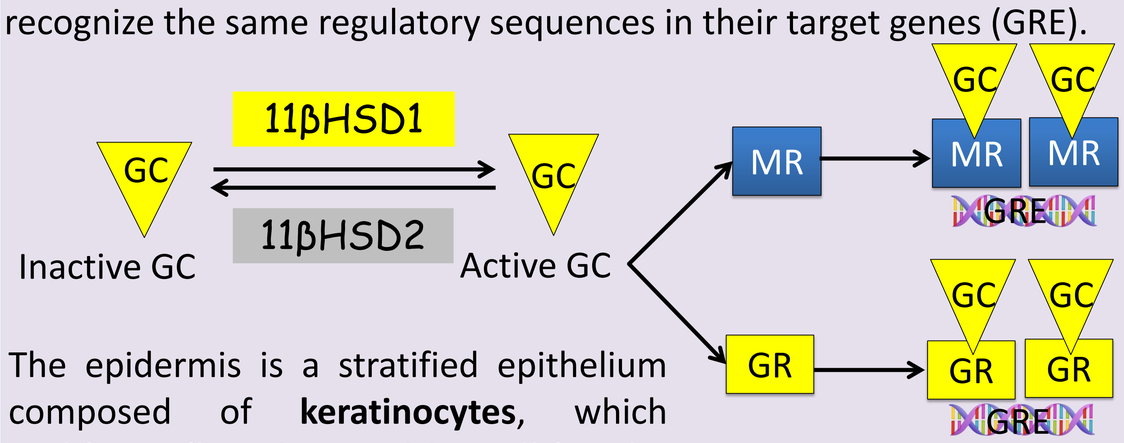
CONTRIBUTION OF GLUCOCORTICOID (GR) AND MINERALOCORTICOID RECEPTOR (MR) IN EPIDERMAL HOMEOSTASIS

Judit Bigas
Lab Paloma Pérez, *Unidad de Modelos animales de patologías cutáneas.*
Instituto de Biomedicina de Valencia (IBV-CSIC), E-46010 Valencia (Spain). Programa de Doctorado en Biotecnología de la UPV.

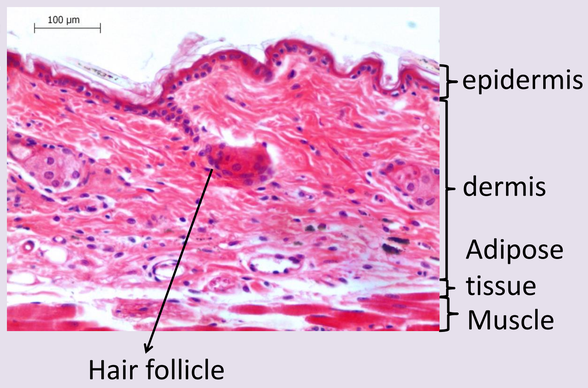
BACKGROUND

It is known that the most effective and widely used therapy for the treatment of skin inflammatory diseases are **synthetic glucocorticoids (GCs)**. Unfortunately, long term use of GCs has unwanted side effects such as skin atrophy. To improve GC based therapies it is essential to understand their mechanism of action.

GCs can bind and activate the **GC receptor (GR)** and the **mineralocorticoid receptor (MR)**, which act as ligand-activated transcription factors that recognize the same regulatory sequences in their target genes (GRE).



The epidermis is a stratified epithelium composed of **keratinocytes**, which proliferate (**keratin 5 positive cells**) in the basal layer (BL). Keratinocytes undergo terminal differentiation in the upper epidermis, where they become flattened, lose their nuclei, and express specialized proteins and lipids forming the stratum corneum (SC), a barrier from the external environment. A specific gene expression program regulates epidermal homeostasis, in which GCs have an important role.



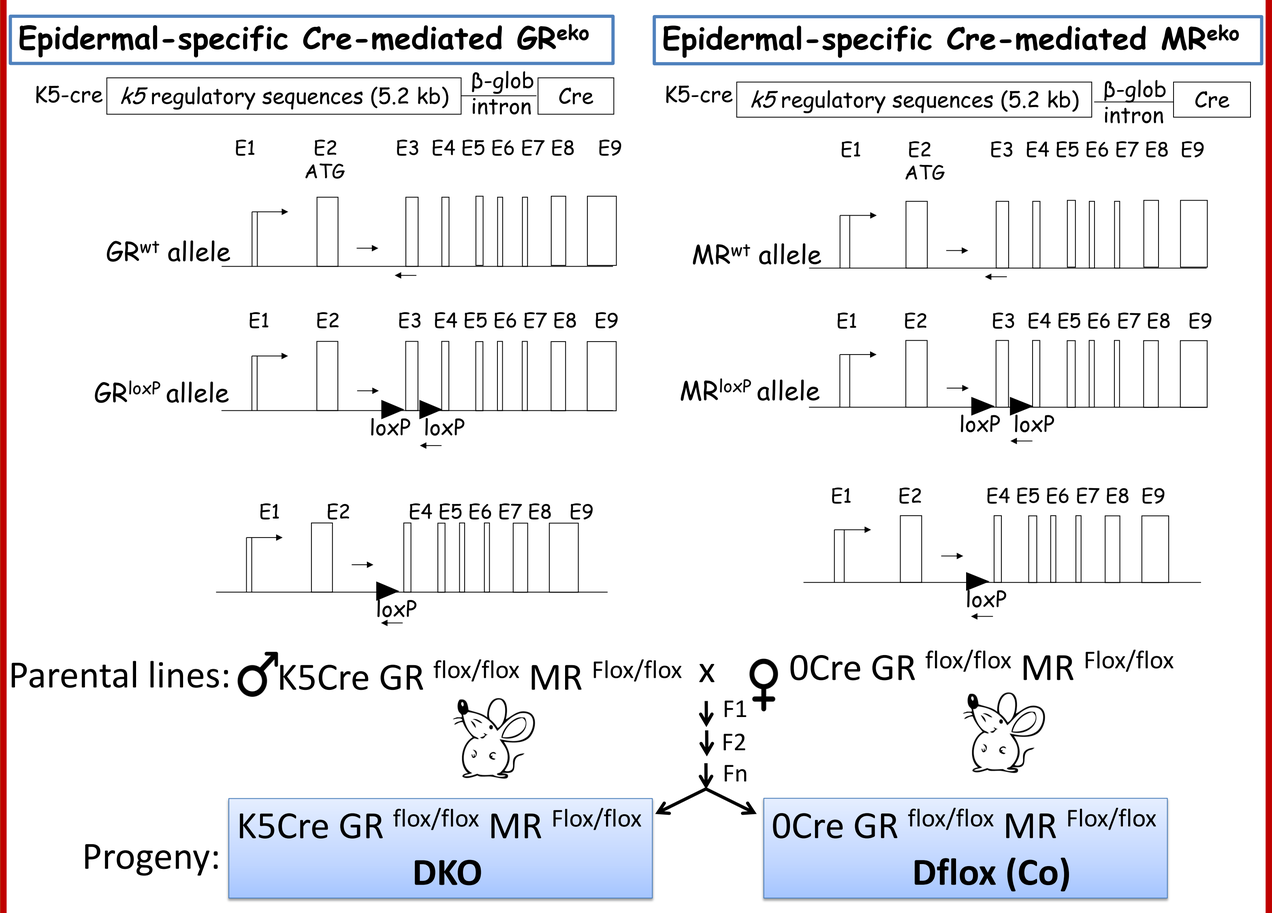
PREVIOUS WORK

Prior research with total inactivation of either GR or MR in mice demonstrated perinatal death. **GR epidermal knock out mice (GR^{eko})** show a skin phenotype resembling key features of atopic dermatitis (AD) at birth and a large inflammatory response to epidermal damage in adulthood (Sevilla et al,. 2013). **MR epidermal knock out mice (MR^{eko})** show minor skin defects at birth. In the adult age, MR^{eko} exhibit increased epidermal proliferation and differentiation both *in vivo* and *in vitro* and a worsened response to inflammatory skin diseases (Boix et al., 2016 under revision).

PROJECT AIM

To generate and characterize mice with epidermal-specific loss of function of both GR and MR (DKO). This will allow us study the consequences of GR/MR inactivation in skin development and function through genetically modified mice and keratinocyte cell lines.

GENERATION OF EPIDERMAL-SPECIFIC GR- AND MR- DOUBLE KNOCK-OUT MICE



IN VIVO STUDIES OF GR^{EKO}/MR^{EKO} or DKO MICE

The main goal is to analyze the skin pathophysiology of DKO adult mice and compare these results with MR^{eko} and GR^{eko} phenotype. Specifically, we will examine aging and inflammatory responses:

- **Aging studies** are important due to the GC implication in epidermal atrophy.
- **Inflammatory response** is studied by epithelial damages by different treatments like sodium dodecyl sulfate (SDS), a skin irritant.

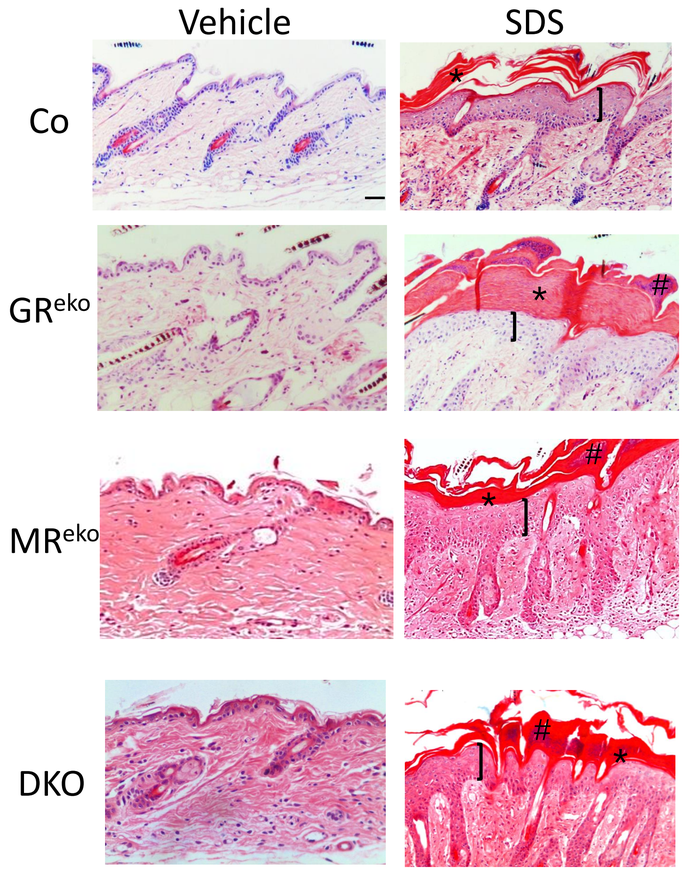


Figure 1) H&E-stained skin sections showing exaggerated stress response in GR^{eko}, MR^{eko} and DKO adult mice after topical SDS treatment (10%, 5 days) compared with control mice (Co). GR^{eko} and MR^{eko} skin sections show an acute inflammatory response. These features are also evident in DKO skin sections, but in addition, DKO mouse skin shows epidermal breakages with inflammatory infiltrates. These results must be quantified to a better understood of each genotype. Bar: 100 µm.
#: epidermal inflammatory infiltrates.
*: hyperkeratosis.
]: epidermal thickening.

IN VITRO STUDIES OF DKO KERATINOCYTE CELL LINE

In these studies we have isolated keratinocytes from dorsal epidermis of GR^{eko}, MR^{eko} and DKO mice and established immortalized keratinocyte cell lines. Our objective is to **characterize** these cell lines including **differentiation** and **proliferation assays** and **response to inflammatory agents**. Rescue experiments in the DKO cell line using either GR or MR can give us additional information about which pathway is involved in GC action. This information may has implications for future design based on GC therapy.

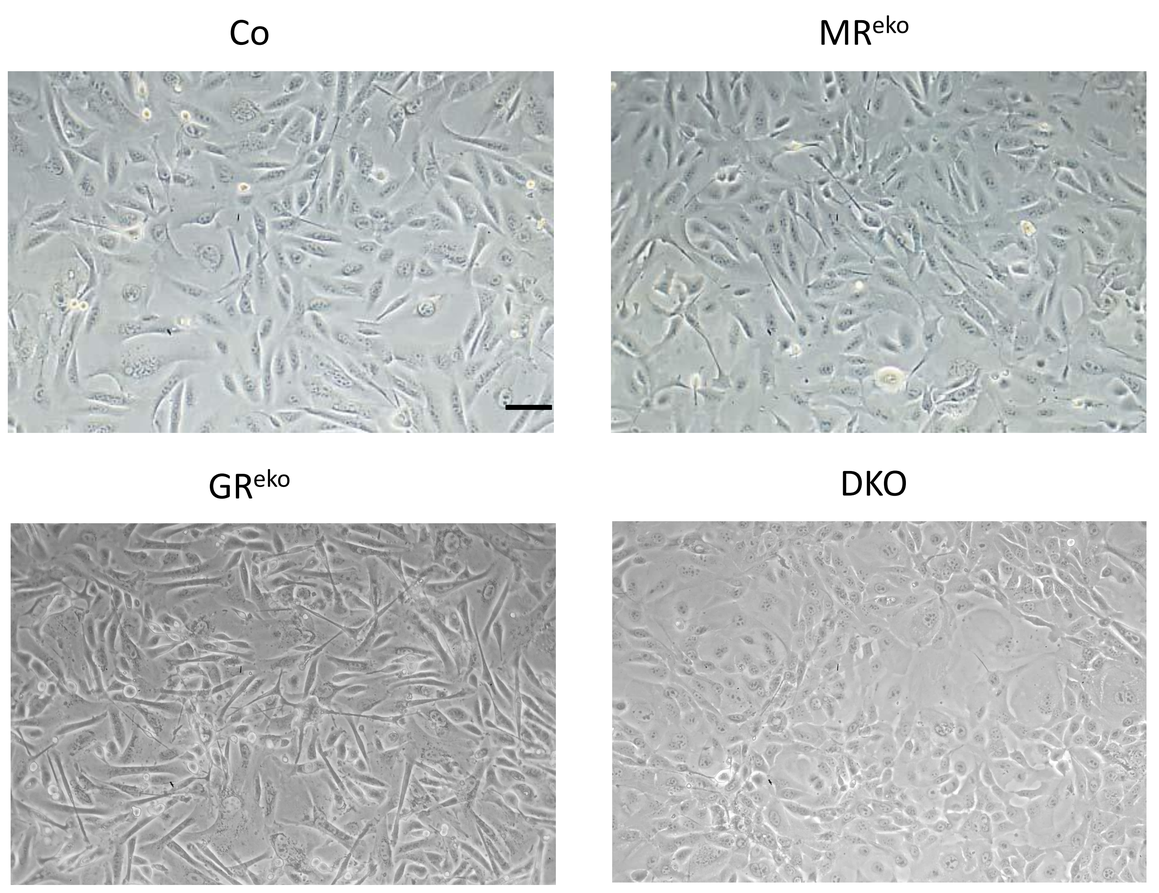


Figure 2) Representative microphotographs show morphological and differential features of Co, GR^{eko}, MR^{eko} and DKO keratinocytes lines. Comparing with Co keratinocytes, GR^{eko} cell line shows an elongated morphology (resembling epithelial-mesenchymal transition, Latorre et al., 2013), while MR^{eko} and DKO cells have a small morphology. However DKO cells are smallest. Scale bar=50µm.

POSSIBLE APPLICATIONS

These results and ongoing experiments using DKO model both *in vivo* and *in vitro* will be important to better understand the role of both GR and MR in skin pathophysiology. Our studies are also intended to establish which **signaling pathways** are involved in GC response in order to discriminate the contribution of each receptor. These studies may contribute to improve **GC-based skin therapies**.

Email: jbigas@ibv.csic.es