

Nanogel-Mediated Protein Replacement Therapy for Autosomal Recessive Congenital Ichthyosis (ARCI)

Roswitha Plank^{1,4}, Katja Obst², Guy Yealland², Marcelo Calderón³, Sarah Hedtrich², Katja Martina Eckl^{1,4}, Hans Christian Hennies^{1,4}

¹Innsbruck Medical University, Center for Dermatogenetics, Div. of Human Genetics
Peter-Mayr-Straße 1, 6020 Innsbruck, Austria
roswitha.plank@i-med.ac.at

²Freie Universität Berlin, Institute of Pharmaceutical Sciences
Königin-Luise-Str. 2+4, 14195 Berlin, Germany
katja.obst@fu-berlin.de; guy.yealland@fu-berlin.de; sarah.hedtrich@fu-berlin.de

³Freie Universität Berlin, Inst. for Chemistry and Biochemistry
Takustrasse 3, 14195 Berlin, Germany
marcelo.calderon@fu-berlin.de

⁴University of Huddersfield, Dept. of Biological Sciences, Dermatogenetics
Queensgate, Huddersfield HD1 3DH, UK
Katja.Eckl@edgehill.ac.uk; h.c.hennies@hud.ac.uk

Extended Abstract

The development of new therapies for rare diseases is often neglected by pharmaceutical companies since there is little profit to be made in this field. For affected patients however, new, effective treatments could greatly improve their quality of life. Moreover, given that there are more than 8,000 diseases that meet the criteria, rare diseases as a whole are frequent and affect many patients.

Autosomal recessive congenital ichthyosis (ARCI) is a rare, monogenic cornification disorder with varying degrees of erythema, epidermal scaling and impaired skin barrier function, leading to transepidermal water loss and frequent infections. To date, treatment schemes like moisturizing creams, bathing and mechanical removing of the skin scales offer only symptomatic relief. Causes of ARCI include a number of genetic mutations, predominantly in genes involved in epidermal differentiation. About 30% of all patients have mutations in the gene *TGM1*, which codes for the enzyme transglutaminase 1 (TGase1). In this study we aim to develop a novel, personalized therapy for ARCI patients with *TGM1* mutations by substituting the defective enzyme TGase1.

We have generated 3D full thickness skin models with keratinocytes and fibroblasts [1] extracted from control persons as well as *TGM1* patients as models for therapeutic testing. The models were comprehensively characterized for gene expression and synthesis of different epidermal markers by immunohistochemistry, real time PCR analysis and Western blot analysis. Our full thickness skin models develop a cornea layer with a functional barrier comparable to the epidermal barrier of the skin, as assessed by Franz-cell analysis. As expected, controls showed a clear difference in barrier activity compared to *TGM1*-patient or *TGM1*-knockdown (KD) 3D skin models, justifying their use as model system to test our novel therapeutic approach.

In order to deliver replacement TGase1 into the keratinocytes of the skin models we encapsulated the enzyme within thermoresponsive poly(N-isopropylacrylamide)-polyglycerol-based nanogels (PNIPAM-dP-NG), which were then applied in varying concentrations and treatment schedules onto the skin models. Interestingly, after application of the TGase1/NG-complex on *TGM1*-patient and *TGM1*-KD models, we observed immunohistochemical staining for TGase1 and greatly improved barrier function compared to the untreated models. These effects could also be shown in a dose dependent manner: The higher the concentration of TGase1 in the used NG the better the barrier of the diseased skin model. Specific activity tests for TGase1 also showed increased enzyme activity in the treated *TGM1*-KD models. Since it has been shown that reduced levels of TGase1 activity are sufficient to prevent the manifestation of diseased skin phenotype [2], the data here

suggests that externally applied TGase1 both enters into keratinocytes and successfully replaces its natural function in TGM1-KD or mutant skin models.

First MTT tests for assessment of toxicity pointed to high biocompatibility of the PNIPAM-dPG nanogel. Further experiments are still needed to determine the best TGase1 dose and the fate of the nanogel following application to skin.

Our findings demonstrate an advanced topical drug delivery system suitable for cutaneous protein replacement as a promising approach towards personalized, causative therapy for ARCI. After further experiments with patient cells, optimization of epidermal delivery and toxicity tests we will adapt our system for other proteins involved in congenital ichthyosis such as the lipoxygenases 12R-LOX and eLOX-3.

References

- [1] K. M. Eckl, T. Alef, S. Torres, et al, "Full-thickness human skin models for congenital ichthyosis and related keratinization disorders," *J. Invest. Dermatol.*, vol. 131, pp. 1938-1942, 2011.
- [2] K. Aufenvenne, V. Oji, T. Walker, et al, "Transglutaminase-1 and bathing suit ichthyosis: molecular analysis of gene/environment interactions," *J. Invest. Dermatol.*, vol. 129, pp. 2068-2071, 2009.