Dissertations

Disease-causing Keratin Mutations and Cytoskeletal Dysfunction in Human Skin: *In vitro* Models and New Pharmacological Strategies for Treating Epidermolytic Genodermatoses

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Jean Christopher Chamcheu from the Department of Medical Sciences, Section for Dermatology and Venereology, Uppsala University Hospital, Sweden defended his thesis on 4 June 2010 at Uppsala University Hospital. The opponent was Professor Torbjörn Egelrud from Umeå University Hospital, Umeå, and the supervisors were Professors Anders Vahlquist and Harshad Navsaria and Drs Hans Törnä and Marie Virtanen.

Epidermolysis bullosa simplex (EBS) and epidermolytic ichthyosis (EI) are rare epidermolytic skin fragility diseases characterized by intra-epidermal blistering, which are largely the result of dominantly-acting mutations in basal (*KRT5* or *KRT14*) and suprabasal (*KRT1* or *KRT10*) keratin genes, respectively. These disorders incurably affect the quality of life of patients and can be lethal in the most severe episodes, posing a disability in the affected families. Despite vast knowledge of the disease pathogenesis, the pathomechanisms of these diseases are not fully understood, and no curative remedies exist for these disorders, presenting a need further to elucidate pathogenic mutations and for the development of novel tools and therapies.

The aim of this thesis was to identify keratin gene mutations in patients with EBS, to develop *in vitro* models as tools for studying the biology of EBS and EI, and to investigate novel pharmacological approaches for treating both diseases.

During these investigations, we discovered novel pI183M and recurrent pE475G pathogenic *KRT5* mutations, as well as single nucleotide polymorphisms in the studied patients with EBS, except for one who did not show any pathogenic keratin mutations (1).

We isolated, established and used cultured primary keratinocytes from EBS patients, to reproduce a genotype–phenotype correlation between clinical severity and cytoskeletal instability *in vitro* using a heat stress model (1).

Human papillomavirus 16 (HPV16)-E6E7 immortalized keratinocyte cell lines were established from three EBS and three EI patients with different degrees of severity, as well as from healthy volunteers (2–4). A small proportion of keratinocyte cell lines derived from severely affected EBS and EI patients exhibited spontaneous keratin aggregates under normal culture conditions (1, 4). However, heat stress significantly induced keratin aggregates in all patient cell lines, with a more dramatic effect observed in cells from patients with severe phenotype (1, 3, 4).

Pretreatment with either of two molecular chaperones, trimethylamine N-oxide dihydrate (TMAO) and sodium 4-phenylbutyrate (4-PBA), reduced the proportion of keratin aggregate-containing keratinocytes in both stressed and un-stressed primary and immortalized EBS and EI keratinocytes cultures, respectively (1–4). The mechanism of action of TMAO and 4-PBA was shown to involve both components of the endogenous chaperone system (heat shock proteins e.g. Hsp70) and the MAPK signalling pathways (3, 4). In addition, MAPK signalling pathways also seemed to be involved in the pathogenesis of EBS. Furthermore, depending on which type of keratin is mutated, 4-PBA up-regulated Hsp70 and *KRT4* (pos-
sibly compensating for mutated \textit{KRT1/S}), and down-regulated \textit{KRT1} and \textit{KRT10}, which could further assist in protecting EBS and EI cells against stress, providing further treatments (4).

In organotypic cultures, the immortalized cells were able to differentiate and form a multilayered epidermis reminiscent of those observed \textit{in vivo}, and the EI cells histologically reproduced the clinical phenotypes of the disease in tissue-engineered epidermis (4).

In summary, novel and recurrent pathogenic keratin mutations have been identified in EBS. Well-characterized primary and immortalized EBS and EI cell lines that functionally reflect the disease phenotype \textit{in vitro} were established. The two pharmacological agents, TMAO and 4-PBA, were shown to be promising candidate compounds for further investigation and development as novel pharmacological treatment of heritable keratinopathies.

\textbf{List of original publications}