CELSA
Central Europe Leuven Strategic Alliance

CELSA - Collaborative research project - Application form - COVER PAGE

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3. Non confidential and public friendly summary (max. 2000 characters)

Molecular basis of keratinopathies

A range of congenital skin blistering diseases, including the notoriously known epidermolysis bullosa simplex (EBS), are linked to mutations in keratins. These proteins form the intermediate filaments present in the cytoplasm of epithelial cells. Mutations in keratins typically cause skin fragility in a dominant way, causing constant suffering of the affected individuals ("Butterfly Children"). At this moment there is no effective treatment for these genetic diseases. This proposal originates from three research laboratories in Leuven, Ljubljana and Prague with pronounced complementarity in technical expertise. Two of us have extensive track record in the intermediate filament research already while the third one excels in the chemical crosslinking technique which is central to this proposal.

Our goal is to investigate the molecular basis of skin fragility caused by keratin mutations, which is an indispensable first step towards a rational design of future therapies. We will investigate the effect of mutations linked to severe EBS phenotypes, such as keratin K5 mutation E475G and keratin K14 mutation R125P, on the filament assembly *in vitro*. These keratins form heterodimers when mixed together, which then associate to tetramers and eventually produce long mature filaments. After *in vitro* co-assembly, chemical crosslinking coupled to mass-spectrometric analysis will be used to structurally analyse the assembly process, towards delineating a specific point at which the assembly is affected by mutations. In parallel, electron microscopy studies using both negative staining and unstained samples in vitreous ice will be performed. In addition, crystallization of truncated heterotetramers will be attempted. Next, we will study the effect of the mutants in cell culture, to be obtained using the induced pluripotent stem (iPS) cell technology. Through epifluorescent microscopy on EGFP-labelled keratins we will document changes in the cytoskeleton caused by these mutations. We will also examine the changes in the mechanical properties of cells by using optical tweezers and live cell imaging. As the result of this multipronged *in vitro* and cell culture study, we expect to fully characterize the effect of several mutations on keratin filament structure and properties. This should serve as a paradigm towards a mechanistical description of keratinopathies, which is an indispensable step towards developing a successful therapy in the future.

4. List 5 key words

Protein structure; disease mechanism; intermediate filaments; keratins; epidermolysis bullosa simplex