Development of 3D epidermal models to understand sacsin function in the skin

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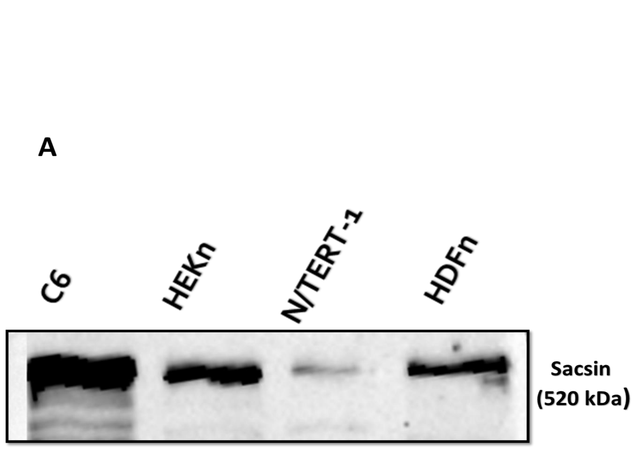
The Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is a rare, early-onset neurological disease characterized by spasticity and lack of muscle coordination, resulting in premature death.

- It is caused by mutations in the *SACS* gene that lead to sacsin loss-of-function, whose role is still unclear.
- Although sacsin is highly expressed in neural cells, it is also expressed in skin cells (Figure 1A), and ARSACS patients show skin alterations.
- To determine the role of sacsin in the skin, we are deleting it from human keratinocyte cell lines N-TERT-1 and HaCaT by a CRISPR/Cas9 approach and creating ARSACS skin cell lines.
- ARSACS cell lines will be used to create 3D skin models, that could later be used to identify molecular mechanisms and markers of disease evolution.
- We are currently optimizing the conditions to produce 3D epidermis from HaCaT and N-TERT-1 cell lines (Figure 1 B,C).



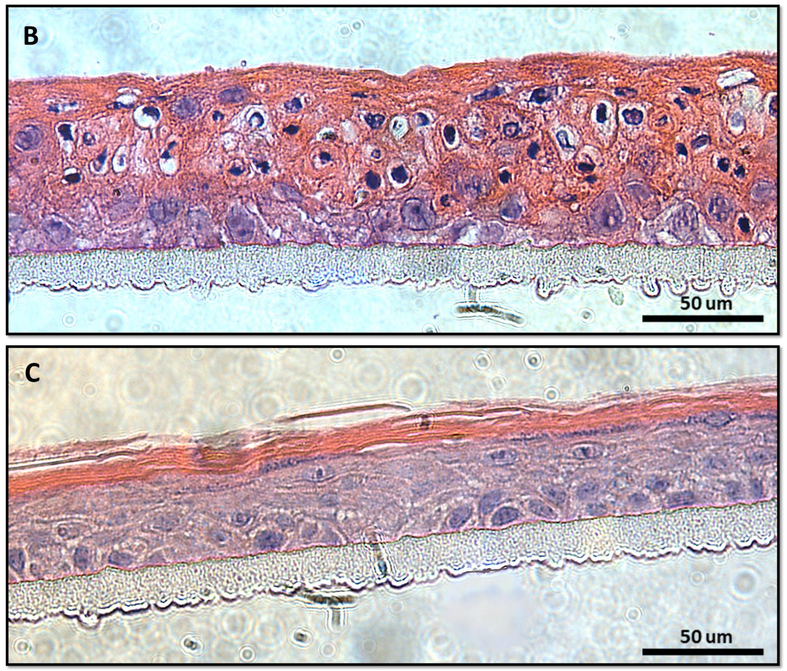


Figure 1 (A, B and C)

Fig. 1A- Primary skin cells (HEKn- normal Human Epidermal Keratinocytes; HDFn- normal Human Dermal Fibroblasts) and the N/TERT-1 cell line express sacsin (C6 glioma cell line is used as positive control)**.**

Fig 1B- Epidermal 3D model using the HaCaT cell line shows a bottom layer that resembles the *stratum basale* of native skin, but the outermost layers show a disorganized architecture without the formation of a *stratum corneum.*

Fig 1C- The N/TERT-1 cell line shows a multilayer architecture that better recapitulates native epidermis with the formation of a *stratum corneum.*

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