Development of new cellular models to identify molecular mechanisms in Hidradenitis Suppurativa

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ABSTRACT / POVZETEK

No satisfactory in-vivo and in-vitro models to recapitulate Hidradenitis Suppurativa (HS) hallmarks have been developed so far. The first transgenic Ncstn KO mice model, engineered after the finding that g-secretase mutations were associated with HS in several families, lacked important HS features such as skin inflammation, abscess formation, fistulas, and scarring. In -vitro, the use of skin explants has helped in the identification of the IL-1 contribution to HS skin inflammation in HS, but this technique depends on skin biopsies availability.

For these reasons we have developed different models to obtain skin cells and skin organoids from Induced Pluripotent Stem cell lines carrying HS-associated mutations

KEYWORDS / KLJUČNE BESEDE

CRISPR/Cas9, Induced Pluripotent Stem Cells, Outer root sheath epithelial cells, Skin Organoids, keratinocytes, sebocytes

1 INTRODUCTION AND RESULTS

Hidradenitis suppurativa (OMIM#142690; HS) is a chronic inflammatory disease involving hair follicles that presents with painful nodules, abscesses, fistulae, and hypertrophic scars, typically occurring in apocrine gland bearing skin [1]. Adequate models reflecting hallmarks of HS pathogenesis are a prerequisite to not only better characterize the molecular activity of genetic mutations in HS, but also to allow the discovery and of therapeutic targets in personalized approaches to cure the disease.

About 10% of HS patients present mutations in three of the four components of the gamma-secretase complex, namely NCSTN, PSEN1 and PSENEN with most of the mutations found in NCSTN [2]. These findings led to the analysis of the NCSTNflox/flox;K5-Cre mice that showed some HS hallmarks such as follicular hyperkeratosis and inflammation [3]. Unfortunately, mica and humans differ not only in hair distribution and hair follicle anatomy, but important genes such as *DCD* identified in a HS family by our consortium doesn't have a homologous in the mouse.

Ex vivo models using patients lesional skins have also been developed [4]. In fact, Vossen et al. [5] cultured punch biopsies from HS patients showing a major contribution of IL-1 in skin inflammation in HS. Moreover, these Authors were able to test different drugs to tame skin inflammation showing the effectiveness of the anti-TNF-a therapy.

Even if this ex-vivo model can be used to test a candidate treatment, specific limitations make this model useless for precision medicine. In fact, different genetic variants seem to cause the disease, so a skin model for each patient (or family) should be developed.

Our Team is developing new cellular models to identify the main biological pathways affected in HS and 3D models to be used to test novel candidate drugs. We are making use of hair follicle epithelial cells isolated from selected patients to build 3D reconstituted immunocompetent skins in collaboration with Dr. Flacher: these models will allow the study of the cross-talk among skin cells and immune cells

At the same time, we have developed skin organoids bearing hair follicles from Induced Pluripotent Stem cells obtained from patients with specific candidate mutations (Figure 1). By using the CRISPR/Cas9 methodology we have been able to correct the candidate mutation and obtain isogenic cell lines differing only for the selected mutation. IPSCs have been differentiated in CD200+/ITGA6+ hair follicle stem cells that could be further differentiated in TP63+/CK14+ keratinocytes or CK7+/MUC1+/PPARG+ sebocytes.

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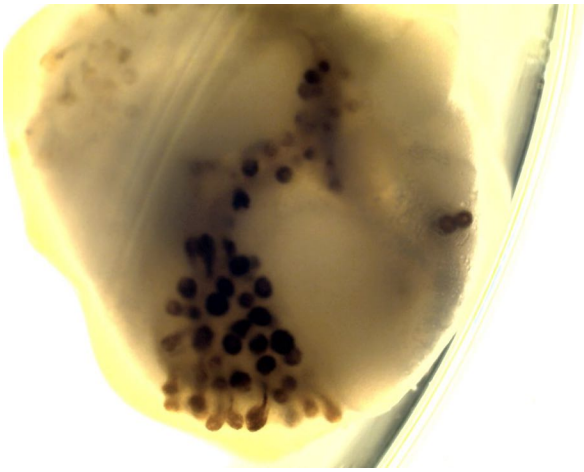

Figure 1: Skin organoids bearing hair follicles from IPSCs

IPSCs obtained from an HS patient with a novel mutation in NCSTN and presenting with HS and DDD were cultivated as described by Lee et al. [6] for 140 days and skin organoids bearing hair follicles obtained from a mutated and corrected clone.

From the skin organoids, thanks to a collaboration with StemCell Technologies, we have been able to isolate and cultivate TP63+/CK14+ keratinocytes (Figure 2)

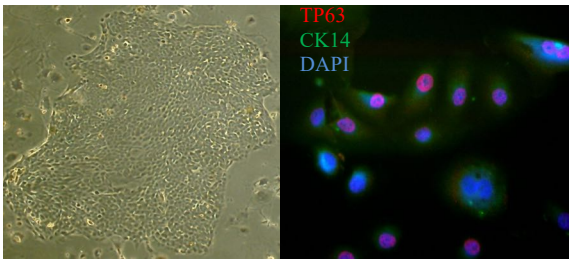

Figure 2: TP63+/CK14+ Keratinocytes isolated from skin organoids

Keratinocytes were obtained after dispase I digestion of skin organoids and cultivated in StemCell Technologies Keratinocyte Medium.

As we have already shown a defect in lysosomes in NCSTN deficient HaCaT cells, we are studying the lysosome structures in TP63+/CK14+ keratinocytes derived from mutated and corrected using lysosomal markers (Figure 3).

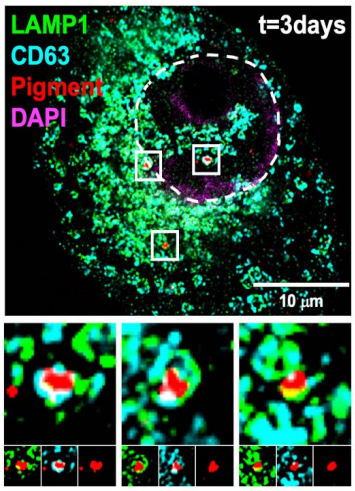

Figure 3: Lysosome distribution in KC obtained from skin organoids

Study of lysosome distribution in mutated and corrected keratinocytes using lysosomal markers CD63, LAMP1 and melanosomes degradation (Pigment)

2 OUTLOOK

Skin organoids will be analyzed by immunofluorescence and immunohistochemistry.

In addition, we plan to understand the activity of *NCSTN* mutation in skin organoids maturation by performing single cell RNA sequencing (Sc-RNAseq). Our hypothesis is that a g-secretase impaired activity skews the differentiation of hair follicle stem cells towards the epithelial keratinocytes. We do expect to see smaller or absent sebaceous glands in our skin organoids and an enlarged population of outer root and inner root sheath keratinocytes.

We plan to carry on the same experiments with IPCSs cell from a patient with a novel *ZNF318* mutation. ZNF318 is involved in Androgen Receptor (AR) signaling [7, 8], that has a major role in sebocytes differentiation [9]. We do expect that a perturbed AR signaling will skew the differentiation of hair follicle stem cells towards the keratinocyte population, still affecting sebaceous gland development.

IPSCs will be differentiated in 2D in CD200+/ITGA6+ hair follicle stem cells and treated to become CK7+/MUC1+/PPARG+ sebocytes (Figure 4) to understand what the activity of the novel ZNF318 mutation is.

IPSCs-derived keratinocytes and sebocytes will be provided to Dr. Flacher's team to build 3D immunocompetent skins.

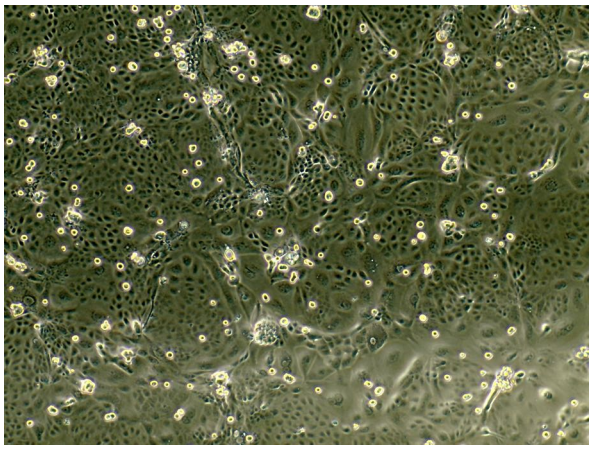

Figure 4: CK7+/MUC1+/PPARG+ sebocytes differentiated from IPSCs

Sebocytes were obtained from IPSCs after 22 days in Sebocyte Culture Medium.

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