Synopsis of Original Research Paper

Examination of 'true' meanings of filaggrin mutation in keratinocytes for various external stimuli

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Previously, we have already successfully generated transgene-free and mutation-free human iPSCs (hiPSCs) from human dermal fibroblasts by using the piggyBac transposon system. Moreover, we successfully differentiated these hiPSCs into epidermal keratinocytes (iKCs; induced keratinocytes).

Incidentally, recent advances in the development of genome editing technologies based on programmable nucleases such as zinc finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease Cas 9 (CRISPR/Cas 9) have substantially improved our ability to make precise changes in the genomes of human cells.

With our established systems of obtaining iKCs from hiPSCs and new technology of programmable nucleases, especially CRISPR/Cas 9 system, we tried to clarify the precise effects of filaggrin gene (*FLG*) mutations in keratinocytes.

A guide RNA that targeted appropriate site of human *FLG* was designed by web-based tool and cloning into the backbone vector of CRIPSR/Cas 9 (h*FLG*-CRISPR/Cas 9). We transfected h*FLG*-CRISPR/Cas 9 into hiPSCs and obtained the several clones of hiPSCs which possessed random mutations in *FLG*. Then, original hiPSC and FLG-mutated hiPSCs were differentiated into epidermal keratinocytes using our established protocols and we obtained the normal iKCs and FLG-mutated iKCs. Under this condition, we can compare the phenotypes of normal and *FLG*-mutaed iKCs of the same genetic background.

Thus, the results obtained from this system should be "true" meanings of *FLG* mutation in keratinocytes and should be important information for the understanding of AD pathogenesis.