Synopsis of Original Research Paper

# Analysis of pathogenesis of KID syndrome by in vivo observation and manipulation of cytoplasmic $Ca^{2+}$ ion concentration

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Connexins (Cxs) make gap-junction/hemichannel that mediate cell-cell/cell-extracellular movement of water-soluble molecules such as calcium ions ($Ca^{2+}$). In some of hereditary diseases caused by mutations in Cxs, marked thickening of corneal layer of the skin occurs (Cx-related keratoderma), resulting in significant cosmetic and functional disadvantages. It is revealed that gain of function mutations that induce hyperactive hemichannels cause Cx-related keratoderma, and that keratinocytes-specific introduction of pathogenic mutant Cx reproduce the hyperkeratosis in mice, indicating that Cx-mutations in keratinocytes are responsible for the hyperkeratosis. However, it is still elusive how hyperactive hemichannels on keratinocytes result in hyperkeratosis. Here, using a mouse model of Cx-related keratoderma in combination with two-photon microscope, we show a close association between hyperkeratosis and distinct dynamics of cytoplasmic $Ca^{2+}$ in Cx-mutant keratinocytes *in vivo*. We made an inducible mouse model of KID syndrome, one of the representative Cx-related keratoderma, by crossing Cx26S17F mice and K14creERT mice. In this model, hyperkeratosis at the footpads of the paws was observed within 2 weeks after the induction of mutation. We then crossed the mice with transgenic mice systemically expressing a $Ca^{2+}$ indicator (GCaMP3) and evaluated the cytoplasmic $Ca^{2+}$-dynamics in keratinocytes throughout the course of disease development. In normal keratinocytes, a transient elevation of cytoplasmic $Ca^{2+}$ preceded the cornification at granular layer uniformly at a single-cell level. In contrast, in keratinocytes with Cx mutations before the formation of hyperkeratosis, patterns of cytoplasmic $Ca^{2+}$ elevation were heterogenous spatiotemporally. Subsequently, we identified intense elevation of cytoplasmic $Ca^{2+}$ in the granular layer just before the formation of hyperkeratosis. Our results not only demonstrate the plausible role of aberrant $Ca^{2+}$-dynamics in the hyperkeratosis of Cx-related keratoderma, but also suggest the importance of strictly regulated $Ca^{2+}$-dynamics for epidermal homeostasis.