

## Development of bioimaging analysis for epidermal cell adhesion molecules

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In this study we attempted to develop a bioimaging analysis for epidermal cell-cell adhesion molecules, desmoglein (Dsg), as well as intermediate keratin cytoskeleton network. We used Dsg3, which is an autoimmune target antigen of pemphigus vulgaris, and keratin 14 and fused them with green fluorescence protein (GFP) or red fluorescence protein (RFP). When these chimeric molecules were introduced into cultured keratinocytes, we could observe time-lapse changes of Dsg3 and keratin as live images without killing or fixing cells. This approach has allowed us to overcome the limitations inherent in establishing a temporal sequence of events from fixed specimens, and to study the assembly and fate of desmosome precursors in living cells during junction assembly. We demonstrated the fate of Dsg3 and keratin after binding of AK23 pathogenic monoclonal IgG antibody. After binding of AK23 IgG, Dsg3 diminished on the cell surface and tended to be internalized into cytoplasm. The insertion of keratin filaments was also obscured and its retraction into cytoplasm was also observed. We are under way to generate transgenic mice with Dsg3-GFP to perform in vivo bioimaging analyses. These new technique for live image of epidermal cell adhesion molecules will provide a valuable tool to understand molecular mechanisms of normal skin, that will in turn contribute to cosmetic science.