3D TEM and SEM-array tomography of Hailey-Hailey Disease human skin biopsies

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Background:
ATP2C1 gene encodes for the secretory pathway Ca2+/Mn2+ pump ATPase type 1 (SPCA1) localizing at the Golgi apparatus. Mutations on the human ATP2C1 gene, causing decreased levels of the SPCA1 expression, have been identified as the cause of the Hailey–Hailey disease (HHD), a rare skin disorder. More than 200 unique mutations have been found, with any hot-spot on the ATP2C1 gene. The HHD patients present raw, blistered areas and lesions, particularly in skin folds where there is moisture and friction. No effective cure exists. Due to reduced expression of functional SPCA1 in HHD patients, altered Ca2+ homeostasis and responsiveness in HHD keratinocytes results in creating an early defect in differentiation process due to a reduced production of involucrin. Keratin expression was also delayed in acantholytic epidermal segments of HHD skin. This results in fragile skin layers. However, no deep investigation at ultrastructural level have been never performed.

Methods:
Advanced gene sequencing has been used to identify mutations on the ATP2C1 gene of HHD patients for this study.
Using volume Scanning Electron Microscopy (SEM)-array tomography we investigated the status of the cell-cell interaction in the epidermis of five different HHD patients. Epoxy resin embedded samples have been sectioned (300nm) using the Powertome-XL ultramicrotome (RMC Boeckler, USA) and serial sections were collected on Kapton tape with an automated ATUMtome collection, and then transferred on Si-wafer support for being imaged in the SEM Gemini 450 (Zeiss, Germany). Sequential images where then managed and over-imposed to generate a 3D model of the cell-to-cell interactions.

Furthermore, using 3D-Transmission Electron Microscopy -tomography we also investigated the alterations in the secretory pathway, with particular focus on the Golgi apparatus, in the keratinocytes (epidermis) and in fibroblasts (dermis). Semi-thin sections (250nm) cut with a UltraCut-UC6 microtome (Leica, Austria) were collected onto formvar-coated copper slot grids and imaged with a Talos L120C Transmission Electron Microscope (TEM) (Thermo Fisher Sc., USA) using motorized tilt-rotate specimen holders. Tilt series datasets were collected at 13500x magnification, using automated data acquisition and image alignment routines as the grids housing the sections were serially tilted over a range of ~130° (±65°) using the microscope Tomo program. Tomograms computed from aligned 2D views were computationally registered and combined in 3D space. All of the above procedures - along with subsequent image segmentation, surface mesh computation/generation, 3-dimensional visualization and quantitative analysis - were performed using the IMOD software package

maintained and distributed by the Boulder Laboratory for 3D Electron Microscopy of Cells at the University of Colorado (Boulder, CO, USA).

Results:
We discovered that interactions among keratinocytes are significantly disrupted, leading to the formation of 'empty spaces' between them. Keratinocytes maintain their tenuous connections primarily through few reamining desmosomes. The overall thickening of the epidermis primarily results from an increase in the number of layers of keratinocytes rather than an expansion of intercellular spaces.
At subcellular level, the Golgi apparatus looks highly swollen, blebbed, and pinched off to larger “bubbles”, rather than vesiculated like previously published in in vitro studies. The overall volume of the Golgi apparatus looks sensitively increased, due to the higher number of the cisternae as well as their volume.
Identification of the heterozygous mutations in the ATP2C1 gene of the 5 patients with HHD revelaed 2 novel mutations (c.2378_2381insTTGT, c.2599A>G), and 3 previously reported nonsense mutation (c.1327C/T, c.1402C>T, c.2554insT).

Conclusions:
We proved a workflow of investigating the overall ultrastructure organization of the human skin in the HHD. For the first time, we observed intracellular morphological organization in the Golgi apparatus of the HHD which is notably different form the previous investigated studies using cell line models. Using SEM array tomography, we also give e deeper view of the general disorganization of the cell-cell contact in the epidermis of the patients. Using combines SEM and TEM methodology we present and feasible approach to deeply investigate poorly studied pathologies, like rare genetic disease.

Keywords:

Hailey-Hailey_Disease; SEM-array-tomography; 3D-TEM- tomography; Golgi_apparatus.

Reference:

N/A