Proteomics of Hair Follicles of whiskers from W mutant mice clarifies KIT restriction on hematopoiesis and melanogenesis

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Overview
MALDI-IMS. Proteomics, W mutant, KIT, Hematopoiesis, Melanogenesis

Introduction
The proto-oncogene c-kit mapped to the white-spotting (W) locus of mice encodes the receptor tyrosine kinase (KIT). The characteristic phenotype of W mutants, which includes anemia and white coat color, can be attributed to the failure of stem cell populations to migrate and/or proliferate effectively throughout development. Here we established a novel strategy to study effects of KIT on hematopoiesis and melanogenesis in whisker follicles which have melanocytes (pigment cells) and cavernous sinus through in situ proteomic analysis.

W mutant mice and the receptor tyrosine kinase c-kit

Methods
Animals
W/mutant mice (gift from Dr. Kazuo, K) at postnatal day (P0) were sacrificed and snap-frozen in liquid nitrogen.

Histopathological and immunohistochemical analysis
10 µm coronal sections through tips of nose were stained with Hematoxylin & Eosin (HE), Fontana - Masson Stain. Immunostaining were performed with anti - MelanA antibody.

Results

1. Histopathological analysis

2. MALDI - IMS

3. Proteomics of whisker follicle

Fig.1 W mutant and structure of KIT

Fig.2 KIT signaling pathway

Fig.3 Histology of whisker follicle of C57BL/6 (black coat color) / a) Whisker follicle b-f) Whisker follicle single section: bar = 250 µm

Fig.4 Materials and Methods

MALDI-IMS
1. The sections were transferred to conductive Indium-Oxide coated glass slides.
2. After washing the sections, on tissue digestion with trypsin was performed with TM-Sprayer (HTX imaging).
3. α-cyano-4-hydroxybenzonic acid (CHA, 10mg/ml) in 70% acetonitrile, 1% trifluoroacetic acid) as a matrix was uniformly deposited on the slides by using TM-Sprayer.
4. The peptides measurements were carried out by using rapiflex (Bruker Daltonics) with the spatial resolution of 50 µm. The mass range: m/z 800-4,000

Shotgun proteomics with the timsTOF Pro
By using timsTOF Pro with nanoElute (Bruker Daltonics) shotgun proteomics was performed with the same tissue sample as IMS and whisker follicles isolated by laser micro dissection. Column used was 25 cm x 75 mm 1.6 µm C18 column. Number of MS/MS ramps was 10PASEF scan.

Data analysis
Obtained mass spectra imaging were visualized with flexImaging 5.0, SCiLS Lab 2019b software. About 2,000 proteins were successfully annotated from coronal sections with Proteinscape 4.0, and database was Swiss-prot. Merged spatial information from MALDI-IMS and identified peptide / proteins information by using ImageID (Bruker Daltonics).

Fig.7 a - c: Segmentation map (bars) = 500 µm, b = 600 µm a' - c' : The images of single peak m/z 1529.739 (bars) = 500 µm, b = 700 µm a'' - c'', H&E staining images (bars) = 500 µm. Square shows the area of whisker follicles.

Fig.8 Isolation of whisker follicles from tissue slides using laser micro dissection and LC - tims MS/MS

Conclusions

Anemia caused by hematopoietic defect, the phenotype of W/W mice was visualized as low intensity of HBA in cavernous sinus by MS/MS and identified Hemoglobin subunit α (HBA).

References