in depth 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 during 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, KIT[1)]

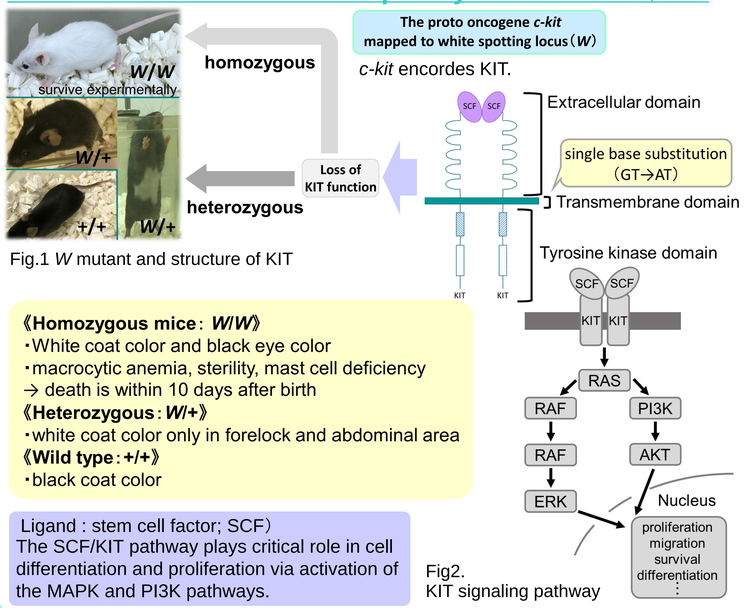


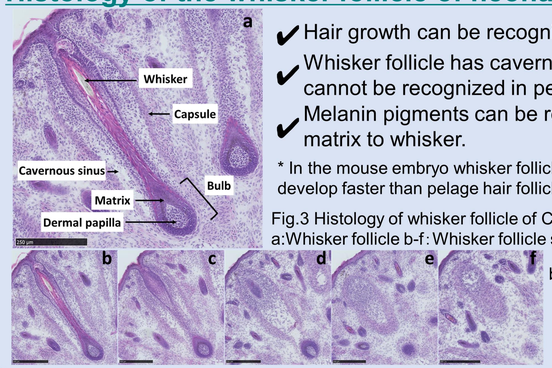
Fig.1 *W* mutant and structure of KIT

Fig2. KIT signaling pathway

《Homozygous mice：*W/W*》
・White coat color and black eye color
・macrocytic anemia, sterility, mast cell deficiency
→ death is within 10 days after birth
《Heterozygous：*W/+*》
・white coat color only in forelock and abdominal area
《Wild type：+/+》
・black coat color

Ligand : stem cell factor; SCF)
The SCF/KIT pathway plays critical role in cell differentiation and proliferation via activation of the MAPK and PI3K pathways.

Histology of the whisker follicle of neonatal mouse



✔Hair growth can be recognized neonatally*.
✔Whisker follicle has cavernous sinus which cannot be recognized in pelage hair follicles.
✔Melanin pigments can be recognized from matrix to whisker.

* In the mouse embryo whisker follicles appear earlier and develop faster than pelage hair follicles.[2)]

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

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.

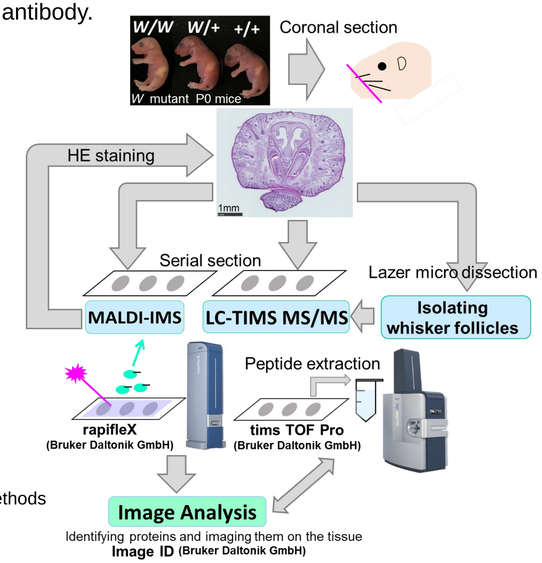


Fig. 4 Materials and Methods

MALDI-IMS

1. The sections were transferred to conductive Indium-Tin-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-hydroxycinnamic acid（CHCA, 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 μm 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).

Results

1. Histopathological analysis

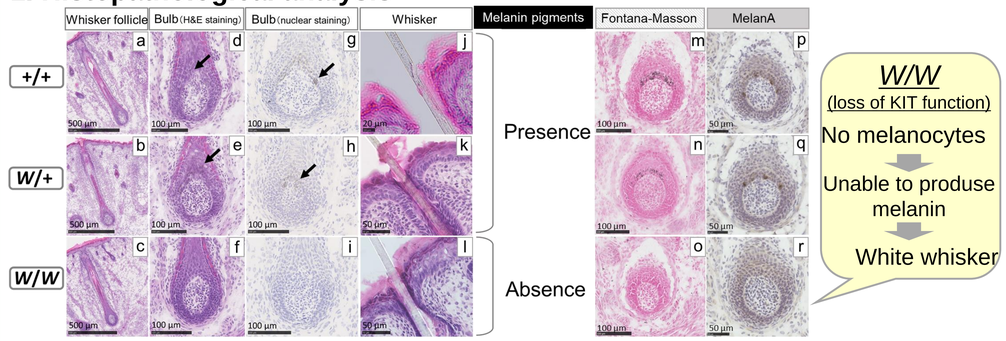
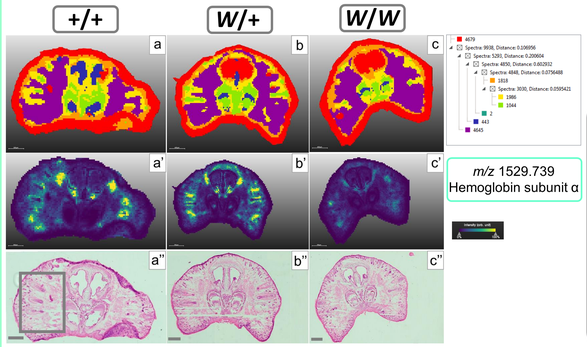


Fig.6 a – l : H&E staining of whisker follicle in *W* mutant mice ※Melanin pigments are indicated by narrows
m – n : Fontana-masson for staining melanin p – r : Immunostaining with the melanocyte marker MelanA

2. MALDI - IMS



W/W has different proteomic pattern from +/+ and *W/+*.

Single peak analysis
Receiver Operating Characteristic (ROC)
・Comparing +/+ with W/W
・Finding the discriminative single *m/z*
***m/z* 1529.739** is specifically distributed in **cavernous sinus of +/+ whisker follicle.**

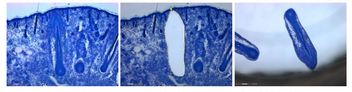
MS/MS ion search
m/z = 1529.739 was identified **Hemoglobin subunit α (HBA).**

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

3. Proteomics of whisker follicle

Focus on **whisker follicles**

The area of one whisker follicle ： about 0.25mm^2



Isolating 3 whisker follicles

Shotgun proteomics

	Protein	Peptide
+/+	217	2983
W/+	265	3846
W/W	196	2955

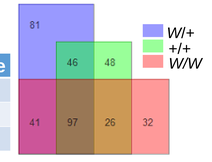


Fig.8 Isolation of whisker follicles from tissue slides using laser micro dissection and LC – tims MS/MS

Conclusions

✔**Anemia caused by hematopoietic defect, the phenotype of *W/W* mice was visualized as low intensity of HBA in cavernous sinus by *In situ* proteomics with MALDI-IMS.**

✔**Proteomics of isolated whisker follicle tissue samples by timsTOF Pro is now ongoing to Identify key proteins of melanogenesis under KIT restriction in whisker follicles.**

References

1) Nocka K, Majumder S, Benoit C, et al. Expression of *c-kit* gene products in known cellular targets of *W* mutations in normal and *W* mutant mice—evidence for an impaired *c-kit* kinase in mutant mice. ***Genes & Development***. 1989, 3 (6), 816-826
2) Davidson P and Hardy MH. The Development of Mouse Vibrissae *in vivo* and *in vitro*. **Journal of Anatomy**. 1952, 86(Pt 4), 342-356